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Abundance and Distribution of Two Species of *Squilla* (Crustacea: Stomatopoda: Squillidae) in the Northern Gulf of Mexico

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ABUNDANCE AND DISTRIBUTION OF TWO SPECIES OF *SQUILLA* (CRUSTACEA: STOMATOPODA: SQUILLIDAE) IN THE NORTHERN GULF OF MEXICO

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ABSTRACT: Stomatopods (mantis shrimps) are predatory benthic crustaceans. Mantis shrimp in the genus *Squilla* are frequent bycatch animals unintentionally collected in conjunction with the shrimp fishery in the Gulf of Mexico (GOM). Their carcasses are discarded instead of being retained for human consumption, fish meal, or other protein-based food products. The size, depth, salinity, and temperature distributions of these species, as well as their abundance based on gender, were examined to gain biological information that would be necessary if a fishery were to develop in the GOM. I collected samples (n = 2,854) of *Squilla empusa* and *Squilla chydaea* in the northern GOM at depths of 1–96 m at 56 stations. *Squilla chydaea* was generally collected in greater abundance and in deeper water compared to *S. empusa*, even though the biomass of *S. empusa* collected in this study was larger than that of *S. chydaea*. For both species, individuals were larger in body length and wet weight in the winter, but more individuals were collected in the summer. Female *S. chydaea* dominated the catch in summer; there was no seasonal difference in sex ratio for *S. empusa*. The potential for commercial harvesting of mantis shrimp in the northern GOM is discussed and compared to other mantis shrimp fisheries.

INTRODUCTION

Mantis shrimp (Crustacea: Stomatopoda) are benthic crustaceans that can be divided into two groups based on the morphology of their raptorial appendages: smashers and spearers (Caldwell and Dingle 1976). Spearer stomatopods represent one of the most commonly found marine fauna collected during trawling activities on the continental shelves in tropical and subtropical regions (Hendrickx and Sanchez-Vargas 2005). Stomatopods are very common in marine waters around the world with around 500 species being described (Muller 1994), and this group is ecologically and economically important. They often prey upon commercially important penaeid shrimps, make up a large portion of the bycatch in the shrimping industry (Hendrickx and Sanchez-Vargas 2005), oxygenate and release nutrients to the benthic habitat by burrowing into the sediment, and are commercially harvested in Europe and Asia.

Stomatopods are fished commercially and used mainly in animal feed and pet food, as biomonitors for toxicity, and for human consumption. Analyses of the nutritional content of *Squilla* have determined the suitable uses and quantities needed for animal feed (Nandeesha et al. 1989, Lekshmy Nair et al. 1991, Reddy et al. 2004). Mantis shrimp in the genus *Squilla* are also used as biomonitors for heavy metals and are useful in determining the pollution levels in waters from environmental disasters and from industrial pollution (Blasco et al. 2002, Storelli and Marcotrigiano 2002). Because the abundance, recruitment, and diversity of stomatopods are negatively correlated with contaminated waters (Risk and Erdmann 2000), monitoring mantis shrimp populations may be an efficient way to estimate pollution levels.

Several species of mantis shrimp are commercially harvested and all are in the family Squillidae. *Squilla mantis* Linnaeus 1758 is commercially fished, consumed by humans in Europe (Abello and Martin 1993), and is of considerable economic importance as the largest crustacean fishery in the Adriatic Sea (5,000 tons/yr) (Froglia and Giannini 1989, Blasco et al. 2002, Chakraborty et al. 2002). *Oratosquilla neap* Latreille 1825, fished off the coast of India, is used in carp diets, poultry feed, and manure (Sukumaran 1987, Nandeesha et al. 1989). *Erugosquilla massavensis* Kossmann 1880 is fished commercially in the Mediterranean (Maynou et al. 2005), and *Kempina mikado* Kemp and Chopra, 1921 is currently fished in the East China Sea (Hamano et al. 1996). *Oratosquilla oratoria* de Haan 1844 is used in sushi and in animal feed, has been highly sought in a commercial fishery off of Japan for the last 40 years, and is the most commercially valuable species in the bottom-trawl fishery with a yearly landing of over 5,000 tons (Yamazaki 1986, Ohtomi et al. 1992, Kodama et al. 2004).

The northern GOM is a main commercial fishing area for penaeid shrimp, and *Squilla* is one of the most abundant bycatch organisms in this region and in the Atlantic Ocean (Wenner and Wenner 1988), and it is thought to prey heavily upon penaeid shrimp. Fishers discard *Squilla* caught in shrimp fisheries and most are dead when discarded (Kesavan Nair and Iyer 1990). *Squilla empusa* Say 1818 and *S. chydaea* Manning 1962 are distributed in the GOM and the Atlantic Ocean (Manning 1969). Both species are similar in morphology of the raptorial appendage and telson spina-

tion, inhabit muddy bottoms, are regularly captured in large quantities in trawls, and are a potential new commercial resource (Rudloe 1971). There is little published information on the ecology of *Squilla* in general (Rockett et al. 1984, Dittel 1991) and in particular of *S. chydaea* (Manning 1969, Perry and Larson 2004). This species has not been cited in reports of extensive collecting from the GOM (Chace 1954) and is believed to be an endemic species within the GOM.

In contrast, *S. empusa* has been studied extensively, with larval descriptions (Morgan and Provenzano 1979) and preliminary growth and distributional data (Chace 1954, Manning 1969, Rockett et al. 1984) being available. Wortham (2001) and Wortham-Neal (2002a, 2002b) have recently studied burrow and habitat preference, reproductive morphology and grooming behavior in *S. empusa*. *Squilla empusa* is benthic, nocturnal, has been found in habitats with penaeid shrimps, is very abundant over sand and muddy bottom (Rudloe 1971, Rockett et al. 1984), and is the most common mantis shrimp in the GOM (Manning 1969, Rudloe 1971). Its distribution has been reported off Texas to Florida in the GOM, in the Atlantic Ocean from New England to Brazil, and in waters near West Africa and South America (Chace 1954, Manning 1969). *Squilla empusa* is also the second most abundant crustacean in numbers and the fourth largest in biomass in the Atlantic Ocean (Wenner and Wenner 1988).

In this paper, I present results on the abundance, distribution, sizes, sex ratio, and habitat of two species of mantis shrimp in the genus *Squilla* in the northern GOM, as well as the first detailed biological information on *S. chydaea*. The objective was to develop and compare the biological and ecological understanding of these mantis shrimp in the GOM, so that if a fishery is established it can be regulated properly.

Materials and Methods

I collected mantis shrimp species off the coasts of Texas, Louisiana, Mississippi, and Alabama in summer and winter months between 1996-2003 on SEAMAP (Southeast Area Monitoring and Assessment Program) cruises aboard the RV *Tommy Munroe*. Some mantis shrimp were also collected in junction with Dauphin Island Sea Laboratory (DISL) in Alabama. Stations were randomly selected prior to the cruise and each was sampled once with a 12.2 m (40 foot) bottom otter trawl with a 2.54 cm (1 inch) mesh cod end; each trawl lasted a maximum of 30 min. Because spearer stomatopods burrow into the substrate, otter trawls likely underestimate their abundance, but can be used to provide relative estimates of sex ratio, size classes, and species diversity (Dittel 1991). Mantis shrimp were only collected from dusk to dawn when they are believed to be out of their burrows.

Bottom water was collected using a Niskin bottle and depth (m), temperature (°C), and salinity (ppt) were measured at each station. The stomatopods collected at each station were sorted by species and preserved in 10% formalin. In the laboratory, the mantis shrimp were measured (carapace length, CL, 0.1 mm) and weighed (WW, 0.01 g). Carapace length was used because Manning (1969), Hamano et al. (1996), and Torisawa et al. (1998) determined that CL is an accurate predictor of total length in spearer mantis shrimp. Gender was determined by identifying the penes at the base of the 8th walking appendage on males and a modified sixth ventral thoracic segment with a seminal receptacle on females (Wortham-Neal 2002a). Length frequencies were recorded for the most abundant species. Stomatopods were also collected in the GOM where water environmental data were not collected and these locations were not included in any calculations. The "winter" season included October through January while the "summer" season included April through July (Abello and Martin 1993). There were months when no collections were made due to weather and boat mechanical problems.

A Shapiro-Wilkes test was used on all data to determine if normality and homogeneity of variance assumptions were met. If normality and variance assumptions were met, linear regression was used to compare CL and WW within a species with gender pooled. If normality and homogeneity of variance assumptions were not met, the data were $\log_{10}$ transformed and if the transformation did not improve normality, then nonparametric Mann-Whitney test (M-W), Spearman's rho correlation, and chi-squared (χ^2) tests were used where appropriate (Siegal and Castellan 1988). I used ANCOVA to examine relationships between CL and WW by gender of each species and then by species with gender pooled, using CL as the covariate. If the parallelism of slopes was statistically upheld ($p > 0.05$), then the adjusted marginal means were compared between genders or between species. If parallelism of slopes was not upheld ($p < 0.05$), then weights between genders or between species could not be compared statistically. Abundances and distribution data were correlated with CL and WW. The data from males and females of the same species were combined if there was not a significant difference in the abiotic factors (salinity, temperature, depth) between gender. Relationships were considered significant if $p < 0.05$.

Results

Three species of Squillidae were collected with *S. empusa* and *S. chydaea* being the most abundant species. Only 3 *Gibbesia neglecta* Gibbes 1850 (formerly *Squilla*; see Manning and Heard 1997) were collected, so this species was not included in any analyses. Of the 55 specifically documented stations where trawling activities resulted in collections of at least one stomatopod, 69% of trawls contained *S. chydaea*, 78% of trawls contained *S. empusa*, and 49% of trawls contained both species. The more numerous species collected was *S. chydaea* (total n = 1,689 from 27 stations, mean =

TABLE 1. *Review of sex ratio (male:female) of different species in the family Squillidae. GOM = Gulf of Mexico.*

Species	***Ratio***	***Reference (Location of Study)***
Oratosquilla oratoria	1 : 1.56	calculated from Kubo et al. 1959 (Japan)
	1 : 1.63	Hamano and Matsuura 1987 (Japan)
	1 : 1.06	calculated from Ohtomi and Shimizu 1988 (Japan)
	1 : 1.21	calculated from Ohtomi et al. 1992 (Japan)
Oratosquilla nepa	1 : 1.65	calculated from Sukumaran 1987 (India)
Kempina mikado	1 : 1.64	Hamano et al. 1996 (China)
Squilla aculeate Bigelow 1893	1 : 1.9	Dittel 1991 (Costa Rica)
Squilla chydaea	1 : 1.11	Wortham this study (GOM)
Squilla empusa	1 : 1.17	calculated from Rockett et al. 1984 (GOM)
	1 : 1.04	Wortham this study (GOM)
Squilla hancocki Schmitt 1940	1.34 : 1	Hendrickx and Sanchez-Vargas 2005 (Gulf of California)
	1 : 1.06	Barbosa-Ledesma et al, 2000 (Mexico)
Squilla mantis	1 : 1.02	calculated from Abello and Martin 1993 (Mediterranean Sea)
	1 : 1.27	Froglia and Giannini 1989 (Adriatic Sea)
Squilla mantoidea Bigelow 1893	1.72 : 1	Hendrickx and Sanchez-Vargas 2005 (Gulf of California)
Squilla panamensis Bigelow 1891	1.23 : 1	Hendrickx and Sanchez-Vargas 2005 (Gulf of California)
Squilla parva Bigelow 1891	1 : 1.2	Dittel 1991 (Costa Rica)
	1 : 1.06	Barbosa-Ledesma et al. 2000 (Mexico)

13.9 ± 0.265) followed by *S. empusa* (total n = 1,198 from 56 stations, mean = 48.9 ± 0.517). Individuals collected at DISL were not included in analyses because of missing abiotic data.

Sex Ratio

The sex ratio of *S. chydaea* was biased toward females in the population with the ratio of males to females being 1:1.11 (n_{males} = 798, $n_{females}$ = 882; χ_1^2 = 4.4, $p < 0.05$); however, the sex ratio of *S. empusa* was equal (1:1.04; n_{males} = 577, $n_{females}$ = 598; χ_1^2 = 0.38, $p > 0.25$). Sex ratios of other spearer mantis shrimp in the family Squillidae are reported in Table 1. For *S. chydaea*, sex ratio varied by season. There were significantly more females than males in winter (M:F; 1:1.25; χ_1^2 = 7.03, $p < 0.01$) even though the sex ratio was nearly equal in summer (M:F; 1:1.05; χ_1^2 = 0.71, $p > 0.25$). For *S. empusa*, the sex ratio was nearly equal in both summer and winter (summer: 1.01:1 sex ratio, χ_1^2 = 0.04, $p > 0.25$; winter: 1:1.23 sex ratio, χ_1^2 = 2.77, $p > 0.05$). See Table 2 for specific frequencies of each gender by species and season. Overall, there were more females than males in *S. chydaea* compared to *S. empusa*, and this female bias was especially noticeable in the winter months.

Morphometric data

For *S. empusa*, there was no difference in the CL-WW relationship between males and females with the slopes being parallel (ANCOVA: parallelism, $F_{1, 1174}$ = 2.41, p = 0.121, WW adjusted means $_{female}$ = 8.93 ± 0.08 g, WW adjusted means $_{males}$ = 8.76 ± 0.08 g). For *S. chydaea*, this relationship differed with the slope of males being steeper (ANCOVA: parallelism, $F_{1, 1688}$ = 11.7, p = 0.001, WW adjusted means $_{female}$ = 5.36 ± 0.03 g, WW adjusted means $_{males}$ = 5.50 ± 0.03 g) (Figure 1).

Individuals of both species had significantly larger CL and WW in winter compared to the summer (Table 3). Carapace length of *S. chydaea* (pooled by gender) was significantly longer than that of *S. empusa* (M-W: *U* = -2.70, p = 0.007) (Figure 2). Even though the mean CL of *S. empusa* was statistically smaller, there was a greater range of CL than *S. chydaea* and more large size class individuals were collected of *S. empusa* than of *S. chydaea* (Table 4). With genders pooled, there was a difference in the CL-WW relationship between *S. chydaea* and *S. empusa*, with the slopes not being parallel, suggesting that the two species grow differently (ANCOVA: parallelism, $F_{1, 2863}$ = 4,144, $p < 0.001$, WW adjusted means $_{chydaea}$ = 5.24 ± 0.04 g, WW adjusted means $_{empusa}$ = 9.12 ± 0.05 g). Otherwise, *S. empusa* has a greater WW than *S. chydaea* when CL was held constant using ANCOVA. Both *S. chydaea* (r^2 = 0.918, $F_{1, 1687}$ = 18,956, $p < 0.001$) and *S. empusa* (r^2 = 0.947, $F_{1, 1173}$ = 21,018, $p < 0.001$) had positive relationships between CL and WW (Figure 3).

The largest WW sample of *S. empusa* collected at any one station was 1,476 g /30 min trawl; *S. chydaea* had 2,341 g/30 min trawl (Table 4). For *S. chydaea*, the station where the largest biomass of stomatopods were caught (i.e., station 3)

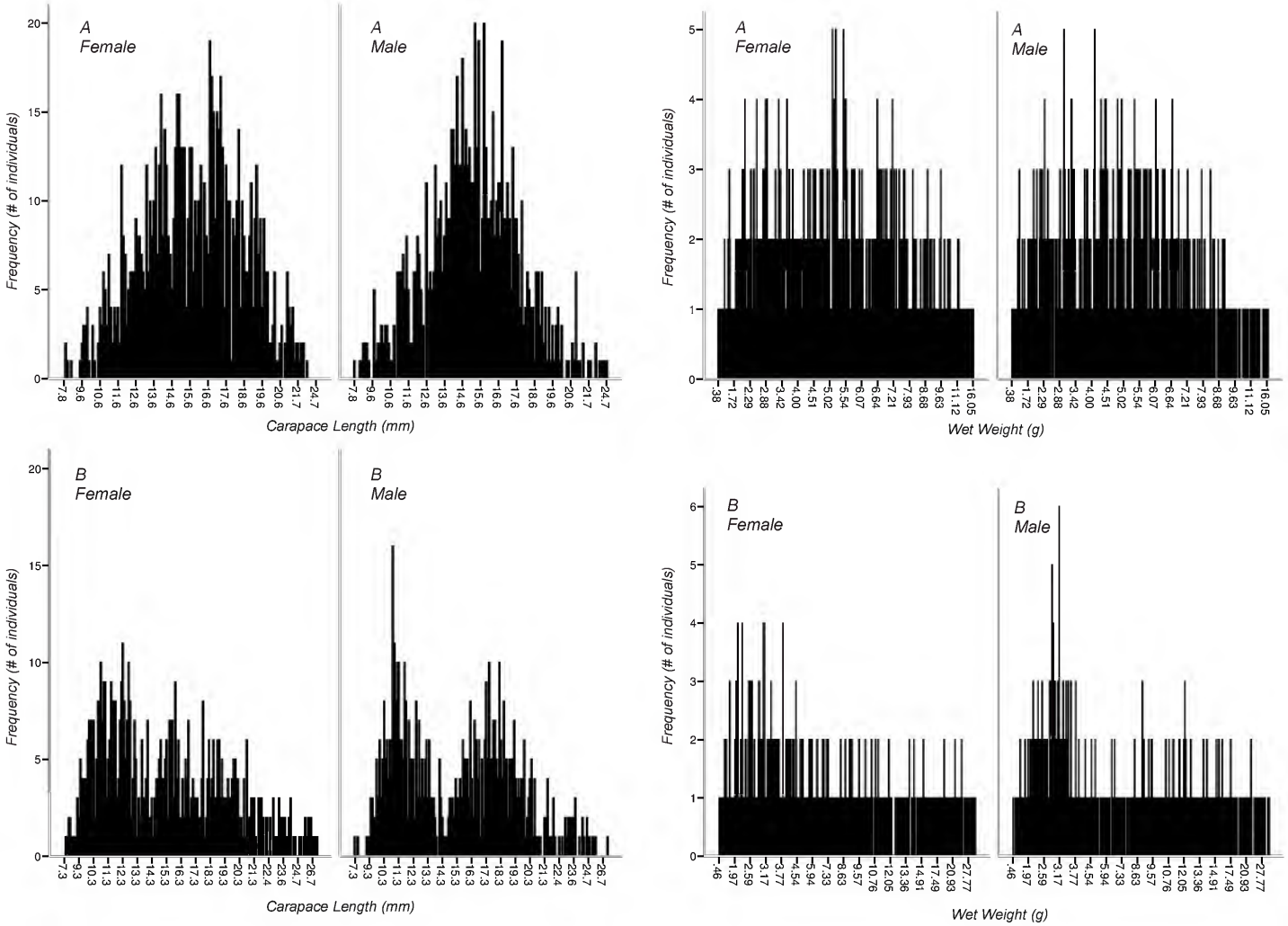

Figure 1.
Carapace length (mm) and wet weight (g) distributions by gender for all Squilla chydaea *(A) and* Squilla empusa *(B).*

was also the station where the largest number of individuals was caught (n = 329). However, in *S. empusa*, this was not the case. The station where the most individuals were caught (station 64; n = 409) had a mean WW of 3.5 ± 0.089 g and carapace length of 11.9 ± 0.093 mm; smaller individuals were collected at this station compared to the station with the largest WW (station 37, n = 125). Overall, both species were larger in the winter while *S. empusa* was larger in WW compared to *S. chydaea*.

Distribution and environmental parameters

Overall, males and females of both species of *Squilla* were distributed equally according to depth, temperature, and salinity (Table 5). *Squilla chydaea* was collected at greater depths than *S. empusa* (M-W: U = -37.9, $p < 0.0001$) (Table 6) and larger individuals were collected in deeper water for both species (*S. chydaea*: Spearman's *rho* = 0.580, $p < 0.0001$; *S. empusa*: Spearman's *rho* = 0.542, $p < 0.0001$). Individuals were collected at significantly different depths between the summer and winter seasons for *S. empusa* (Table 3E) and for *S. chydaea* (Table 3J). *Squilla empusa* apparently occurs in shallower waters in the winter, whereas *S. chydaea* apparently occurs in deeper waters in the winter. For both species, there were no significant difference among the depths at which males and females were collected (Table 5D).

Squilla empusa was collected in significantly warmer water than *S. chydaea* (Table 6) and larger individuals were collected in colder water (M-W: U = -33.5, $p < 0.0001$) (*S. chydaea*: Spearman's *rho* = -0.459, $p < 0.0001$; *S. empusa*: Spearman's *rho* = -0.493, $p < 0.0001$). Individuals were collected at significantly different temperatures between the summer and winter seasons for both species (Tables 3D and 3I). There were no significant difference among the temperatures where males and females were collected for *S. chydaea*; however, *S. empusa* males and females were collected at significantly different temperatures, even though the temperature difference was <1°C (Table 5C). For *S. empusa*, females were collected from significantly higher temperatures than males (Table 5); however, the mean temperature difference of <1 °C between males and females is not likely to be biologically significant.

TABLE 2. *Seasonal data of carapace length (CL, mm) and wet weight (WW, g) of* Squilla empusa *and* Squilla chydaea.

Species, Season, Sex, Measurement, % of total n	n	Mean	Median	Range by gender	Biomass Sum	% Total Biomass Sum
Squilla chydaea:						
Winter						
Female: (16.9%)						
WW	286	5.83	5.85	0.54 – 14.47	1,669	18.2
CL	286	16.3	16.8	7.9 – 22.0	–	–
Male: (13.4%)						
WW	226	5.58	5.13	1.0 – 17.27	1,262	13.8
CL	226	15.9	14.8	8.5 – 23.9	–	–
Totals: (30.3%)						
WW	512	5.72	5.48	0.54 – 17.27	2,931	32.0
CL	512	16.1	16.4	7.9 – 23.9	–	–
Summer						
Female: (35.7%)						
WW	603	5.49	5.13	0.38 – 16.60	3,313	36.2
CL	603	15.5	15.3	7.9 – 23.3	–	–
Male: (34.0%)						
WW	574	5.09	4.89	0.45 – 19.14	2,920	31.9
CL	574	14.9	15.0	7.8 – 24.7	–	–
Totals: (69.7%)						
WW	1,177	5.30	4.96	0.38 – 19.14	6,233	68.0
CL	1,177	15.2	15.2	7.8 – 24.7	–	–
Overall Totals						
Female: (52.6%)						
WW	889	5.60	5.26	0.38 – 16.60	4,982	54.4
CL	889	15.7	15.7	7.9 – 23.3	–	–
Male: (47.4%)						
WW	800	5.23	4.95	0.45 – 19.14	4,182	45.6
CL	800	15.2	15.2	7.8 – 24.7	–	–
Totals: (100%)						
WW	**1,689**	**5.42**	**5.13**	**0.38 – 19.14**	**9,164**	**100**
CL	**1,689**	**15.5**	**15.4**	**7.8 – 24.7**	**--**	**--**
Squilla empusa:						
Winter						
Female: (12.3%)						
WW	145	9.28	8.72	0.92 – 33.39	1,346	12.9
CL	145	15.9	15.9	7.9 – 26.0	–	–
Male: (10.0%)						
WW	118	9.47	8.32	1.63 – 24.52	1,117	10.7
CL	118	16.1	16.3	9.6 – 24.2	–	–
Totals: (22.4%)						
WW	263	9.36	8.42	0.92 – 33.39	2,463	23.7
CL	263	16.0	16.0	7.9 – 26.0	–	–
Summer						
Female: (38.6%)						
WW	453	8.74	5.74	0.46 – 40.67	3,960	38.1
CL	453	15.2	14.5	7.5 – 28.4	–	–
Male: (39.1%)						
WW	459	8.65	7.81	0.58 – 41.09	3,970	38.2
CL	459	15.2	15.4	7.3 – 28.4	–	–
Totals: (77.6%)						
WW	912	8.70	6.53	0.46 – 41.09	7,930	76.3
CL	912	15.2	14.9	7.3 – 28.4	–	–
Overall Totals						
Female: (50.9%)						
WW	598	8.87	6.36	0.46 – 40.67	5,306	51.1
CL	598	15.3	14.9	7.5 – 28.4	–	–
Male: (49.1%)						
WW	577	8.82	7.96	0.58 – 41.09	5,087	48.9
CL	577	15.4	15.6	7.3 – 28.4	–	–
Totals: (100%)						
WW	**1,175**	**8.85**	**6.98**	**0.46 – 41.09**	**10,393**	**100**
CL	**1,175**	**15.4**	**14.8**	**7.3 – 28.4**	**--**	**--**

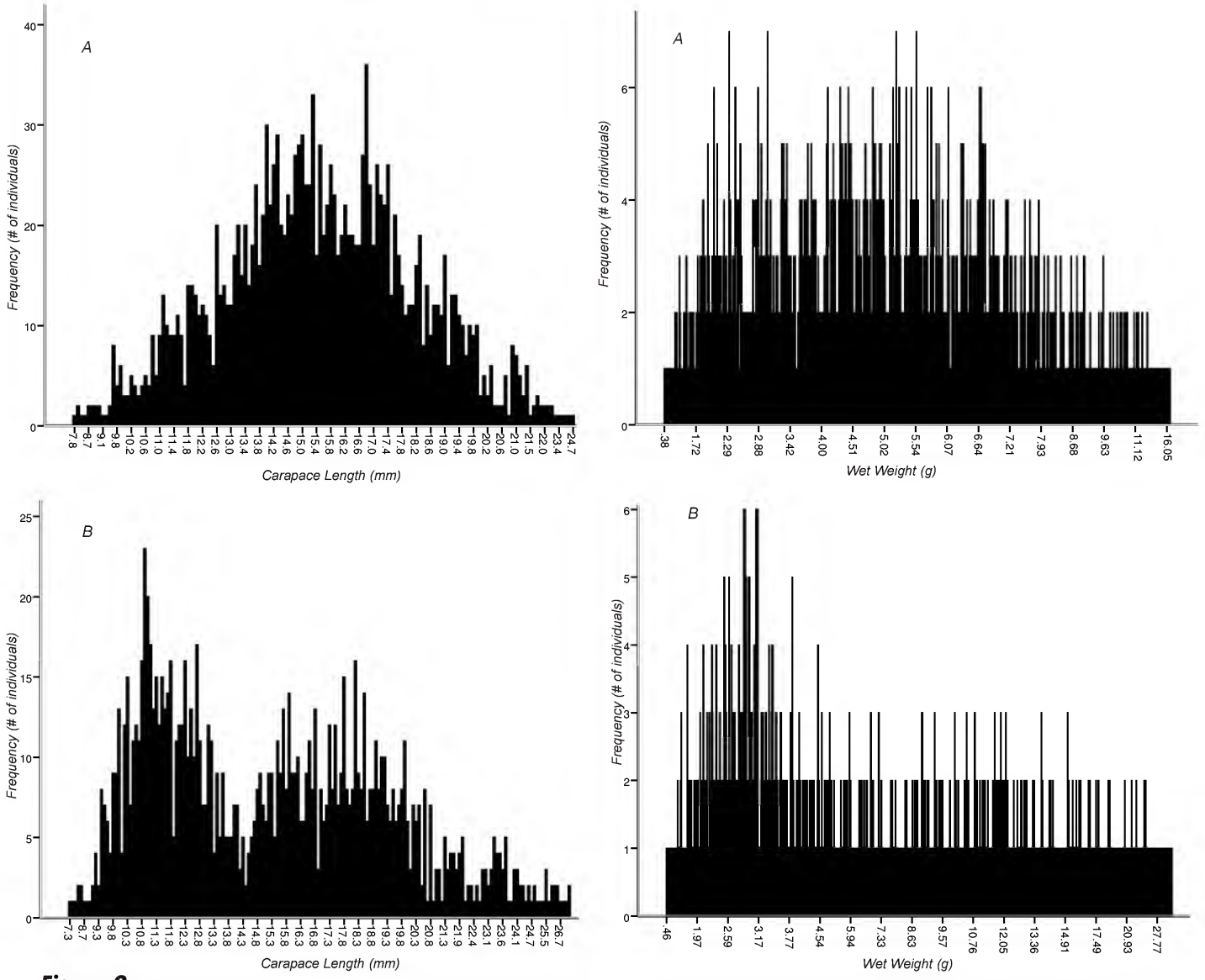

Figure 2.
Carapace length (mm) and wet weight (g) distributions for all Squilla chydaea *(A) and* Squilla empusa *(B).*

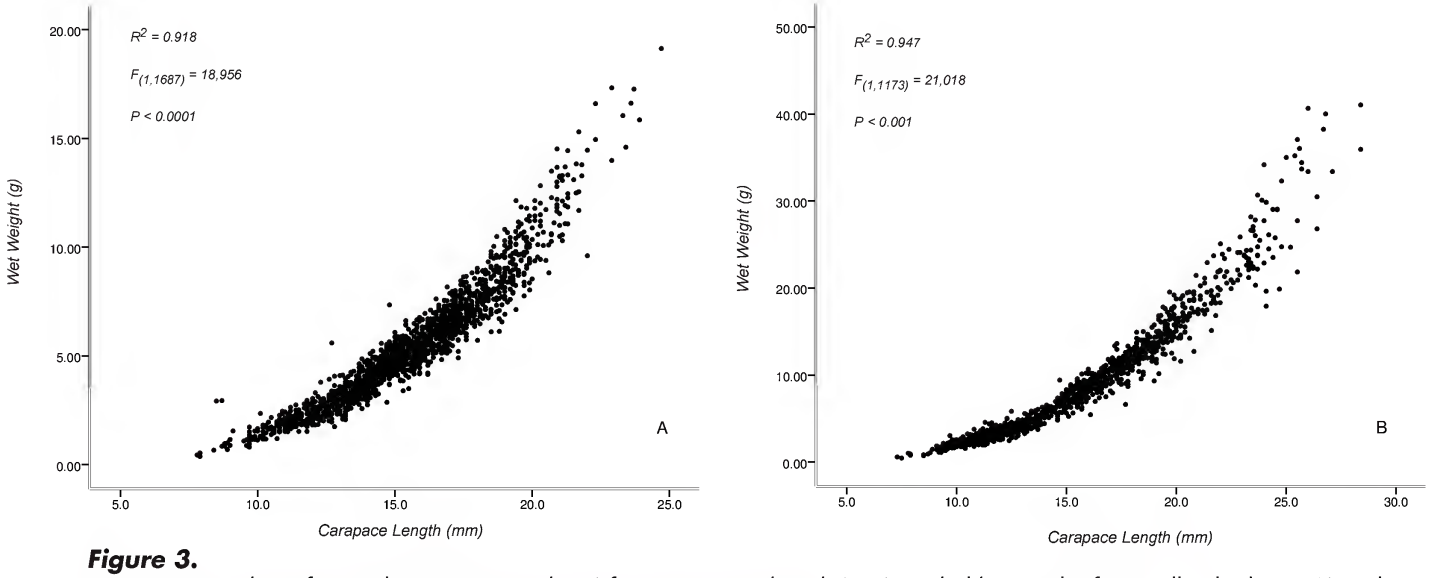

Figure 3.
Regression analyses for predicting wet weight (g) from carapace length (mm) pooled by gender for Squilla chydaea *(A) and* Squilla empusa *(B). Note different axis values for each species.*

Squilla chydaea was collected in significantly higher salinity waters than *S. empusa* and larger individuals were collected in higher salinity waters (Mann Whitney $U = -21.5$, $p < 0.0001$) (*S. chydaea*: Spearman's *rho* = 0.255, $p < 0.0001$; *S. empusa*: Spearman's *rho* = 0.435, $p < 0.0001$). The range, mean, and most common salinities between the two stomatopods were *S. chydaea*: 31–40 ppt, 36.5 ppt for both males and females, and 35-37 ppt, respectively; *S. empusa*: 32–40 ppt, 36.0 for both males and females, and 35–36 ppt, respectively. Individuals were collected at significantly different salinity between the summer and winter seasons for both species (Tables 3C and 3H). Males and females were collected from waters of similar salinity year round (Table 5B).

Squilla chydaea was collected at a rate of 73.6 individuals/station in the summer compared to 45.7 individuals/station in the winter (Table 2; $\chi_1^2 = 6.47$, $p < 0.02$). Likewise, *S. empusa* was also collected at a higher rate in the summer of 41.9 individuals/station compared to the winter of 6.68 individuals/station (Table 2; $\chi_1^2 = 25.5$, $p < 0.0005$). Trawls in the summer yielded more productive harvests for both species; the per trawl WW of *S. empusa* and *S. chydaea* in the summer was 364.5 g and 390.1 g, respectively, and 62.5 g and 261.4 g, respectively, in winter. For the 2 species combined, a mean trawl in the summer would harvest 754 g whereas a trawl in the winter would harvest 324 g (Table 2). Overall, both species had genders that were equally distributed by all environmental parameters, were collected in higher frequencies in the summer and had summer trawls that yielder larger total WW, and had larger individuals that were collected at deeper, colder, higher salinity waters. *S. chydaea* was found in colder, deeper, higher salinity waters than *S. empusa*.

Discussion

The present study represents the first detailed study and data on *S. chydaea* as well as an analysis of important biological similarities and differences of the two most abundant mantis shrimp in the GOM. Both *Squilla* species were collected in high frequencies in the GOM, often together, with more individuals of *S. chydaea* being collected. Overall, compared to *S. empusa*, *S. chydaea* had more females than males, a smaller WW, and lived in deeper, colder, higher salinity waters. Both species were collected in higher abundances in the summer, were larger in the winter and in deeper, colder, and higher salinity waters, and had genders that were equally distributed by environmental parameters.

Similar to research on other spearer mantis shrimp, the sex ratio of *S. empusa* also differs between winter and sum-

TABLE 3. *Seasonal abiotic and size data for collections of* Squilla empusa *(A-E) and* Squilla chydaea *(F-J) with sexes pooled. U = Mann-Whitney Test.; * indicates a significant difference; IQR is the interquartile range.*

Measurement	Median (IQR)	Statistic *U*	p
Squilla empusa			
(A) Carapace length (mm)	Summer: 14.1 (6.3) Winter:17.7 (6.5)	-3.64	<0.0001*
(B) Body mass (g)	Summer:5.64 (8.34) Winter:10.6 (10.5)	-3.50	<0.0001*
(C) Salinities (ppt)	Summer: 35.5 (1) Winter:34.7 (4)	-12.4	<0.0001*
(D) Water temperature (°C)	Summer:23.2 (3.2) Winter:24.4 (1.6)	-5.91	<0.0001*
(E) Depth (m)	Summer:21.0 (7.0) Winter:13.0 (19.0)	-15.0	<0.0001*
Squilla chydaea			
(F) Carapace length (mm)	Summer:15.2 (3.4) Winter:16.3 (4.1)	-6.27	<0.0001*
(G) Body mass (g)	Summer:4.96 (3.29) Winter:5.47 (3.74)	-3.35	0.001*
(H) Salinities (ppt)	Summer: 36.1 (1.0) Winter:37.0 (0.0)	-7.55	<0.0001*
(I) Water temperature (°C)	Summer:21.1 (2.4) Winter:16.8 (2.2)	-29.4	<0.0001*
(J) Depth (m)	Summer:47.0 (24.0) Winter:66.0 (18.0)	-17.7	<0.0001*

TABLE 4. *Wet weight (WW, g) and carapace length (CL, mm) of* Squilla chydaea *and* S. empusa. *"Largest trawl" refers to the station in which the largest biomass of a species of mantis shrimp was collected.*

Measurement	***Squilla chydaea***	***Squilla empusa***
Number of individuals in analyses	1,689	1,175
Total WW of all stations	9,163	10,393
Mean (± se) individual WW	5.42 ± 0.065	8.85 ± 0.204
Median individual WW	5.13	6.98
Mean (± se) individual CL	15.5 ± 0.066	15.4 ± 0.121
Median individual CL	15.4	14.8
Number of individuals collected in 30-min largest trawl	329	125
Largest biomass (g) collected/ 30-min (i.e. largest trawl)	2,341	1,476
Mean (± se) individual WW in largest trawl	7.11 ± 0.150	11.80 ± 0.335
Median individual WW in largest trawl	6.60	11.35
CL mean (± se) in largest trawl	17.0 ± 0.120	17.9 ± 0.178
CL median in largest trawl	16.8	17.8

mer. For the entire year, the sex ratio was 1:1.04, but it was 1:1.01 in the summer and 1:1.23 in the winter. Sex ratios of spearer stomatopods, including *S. empusa* (Rockett et al. 1984), vary during the spawning season (usually in the summer months) because females remain in their burrows to care for egg masses and are less likely to be captured, leading to a more equal sex ratio. However, males actively search for a new cavity after giving this resource to the female in the breeding season, thus allowing males to be collected (Ohtomi et al.1989, Torisawa et al. 1998, Maynou et al. 2005). For example, *O. oratoria* and *Hemisquilla californiensis* Stephenson 1967 (Basch and Engle 1988) have a sex ratio of more males compared to females during the breeding months when the females are in the burrows and males may be actively searching for a burrow, but a biased sex ratio towards more females (1:5.24) in the non-breeding months when females are more likely to be out of their burrow (Hamano and Matsuura 1987).

While abiotic factors such as salinity, temperature, and depth influenced what *Squilla* species were collected (Abello and Martin 1993, Jesse 1996, Perry and Larsen 2004), interspecific competition for resources may also influence *Squilla* distributions (Dingle and Caldwell 1975). Both *Squilla* in this study likely live in similar burrow types (both spearers living in muddy bottoms and collected in same trawls) and eat similar prey (both have similar raptorial appendages; Manning 1969), suggesting that they may compete for resources and live in different environmental niches.

In this study, larger *S. chydaea* were more likely to be females than males. However, in *S. empusa*, there were no differences between male and female CL, which is similar to the findings of Rockett et al. (1984). No difference between CL of pooled males and females were found in the spearers *O. oratoria* (Torisawa et al. 1998), *O. neap* (Sukumaran 1987), *S. mantis* (Abello and Sarda 1989, Abello and Martin 1993), and *K. mikado* (Hamano et al. 1996). In contrast, males were larger than females in *S. parva* and *S. aculeata* (Dittel 1991), and body sizes of both species were smaller during the winter months compared to summer months. However, in my study where individuals were larger (CL and WW) in the winter, the size difference between seasons may be biased due to females residing in burrows in summer and not being collected as frequently.

Two main species of stomatopods are harvested commercially worldwide, *O. oratoria* in Japan and *S. mantis* in Europe, and both are comparable to the mantis shrimp in this study. For example, like *Squilla* in the GOM, *Oratosquilla* has differences between the seasons in total landings and individual size (Ohtomi et al. 1989, Ohtomi and Shimizu 1996). *Squilla mantis* is very similar to the two species of stomatopods in this study by being collected mostly in night trawls (Froglia and Giannini 1989) and having seasonality in total landings (Abello and Martin 1993). Other similarities between *S. mantis* and *S. empusa* include males and females with similar CL (Maynou et al. 2005), smaller individuals located in shallower waters (Abello and Martin 1993), and

TABLE 5. *Characteristics of female and male related to size and abiotic factors for* Squilla empusa *and* Squilla chydaea. *U = Mann-Whitney Test.; * indicates a significant difference; IQR is the interquartile range.*

Measurement	Species	Median (IQR)		Statistic *U*	p
		Males	Females		
(A) Carapace length (mm)[A]	*S. empusa*	15.2 (7.3)	14.1 (6.7)	-0.60	0.55
	S. chydaea	15.2 (3.2)	15.7 (4.0)	-4.33	<0.0001*
(B) Salinity (ppt)[B]	*S. empusa*	35.5 (1.0)	35.0 (1.0)	-1.75	0.08
	S. chydaea	37.0 (1.0)	36.3 (1.0)	-0.011	0.99
(C) Water temperature (°C)[C]	*S. empusa*	23.2 (2.3)	24.0 (2.3)	-2.17	0.03*
	S. chydaea	19.6 (1.8)	20.1 (2.3)	-0.96	0.34
(D) Depth (m)[D]	*S. empusa*	21.0 (7.0)	21.0 (7.0)	-0.64	0.52
	S. chydaea	56.0 (21.0)	47.0 (21.0)	-1.34	0.18

[A] The range of carapace length for male and female in *S. empusa* was 7.3–28.4 mm and 7.5–26.8 mm, respectively; *S. chydaea* was 7.8–24.7 mm and 7.9–23.3 mm, respectively. The range of body mass for male and female in *S. empusa* was 0.58–41.09 g and 0.46–40.67 g, respectively; *S. chydaea* was 0.45 19.14 g and 0.38–16.6 g, respectively. [B] The range of salinity for both males and females of *S. empusa* was 32.0–40.0 ppt; *S. chydaea* was 35.0–40.0 ppt. [C] The range of temperatures for both male and female of *S. empusa* was 20.8–25.5 °C; *S. chydaea* was 16.8–23.5 °C. [D] The range of depths for both males and females of *S. empusa* was 1–57 m; *S. chydaea* was 21–96 m.

TABLE 6. *Depth (m) and temperature (°C) distributions of* Squilla chydaea *and* Squilla empusa.

Measurement	***Squilla chydaea***	***Squilla empusa***
DEPTH:		
Depth range of species	21-96	1-57
Mean (± se) depth of species	48 ± 0.341	26 ± 0.298
Median depth of species	48	21
Mean (± se) depth of males	49 ± 0.492	26 ± 0.420
Mean (± se) depth of females	47 ± 0.472	26 ± 0.423
Median depth of males	56	21
Median depth of females	47	21
Most common catch range (m)	30-60	10-40
Median depth caught in summe	47	21
Median depth caught in winter	66	13
TEMPERATURE:		
Temperature range of species	16.8–23.5	21.0–25.0
Mean (± se) temperature of species	20.2 ± 0.049	23.1 ± 0.039
Median temperature of species	20.1	23.5
Mean (± se) temperature of males	20.1 ± 0.067	23 ± 0.055
Mean (± se) temperature of females	20.1 ± 0.070	23 ± 0.054
Median temperature of males	19.6	23.2
Median temperature of females	20.1	24.0
Most common catch range (°C)	18–22	21-24
Median temperature caught in summer	21.1	23.2
Median temperature caught in winter	16.8	24.4

high abundance in collections <60 m (Froglia and Giannini 1989, Maynou et al. 2005; Table 7). *Squilla mantis* is similar to *S. chydaea* in that there not being a relationship between individual size and depth (Maynou et al. 2005), they are collected at depths up to176 m (Abello and Sarda 1989), and the sex ratio is variable between seasons (Froglia and Giannini 1989, Maynou et al. 2005).

While there is no current fishery or management in the United States for stomatopods, some predictions about a mantis shrimp fishery in the GOM may be made using data from current mantis shrimp fisheries in other countries. For example, the type of net used and vessel size has been reported to influence the catch of *O. oratoria* (Ohtomi et al. 1992, Tokai et al. 1997). Several population dynamics studies have

TABLE 7. *Review of depth range where* Squilla empusa *has been most abundantly collected.*

Reference	Depth (m)	Location of Collections
Hildebrand (1954)	35-42	Gulf of Mexico—Louisiana coast
Camp (1973)	18	Gulf of Mexico—Florida coast
Rockett et al.[A]	9-16	Gulf of Mexico—Texas coast
Wenner and Wenner (1988)[B]	< 20	Atlantic Ocean—Southeast
Wortham (present study)[C]	15-60	Gulf of Mexico—Northern coast

[A] Total depth range 5-86 m with minimum collections at 64-86 m, [B] Collections made only in the daytime, [C] Off the coasts of Texas, Louisiana, Alabama, Mississippi, and Florida

been conducted on *S. mantis* (Badia et al. 1976, Abello and Martin 1993) and determined that gravid females are more valuable at market than males and non-gravid females (Abello and Martin 1993). In *S. empusa*, gravid females were collected only in the summer months (Wortham-Neal 2002b) and stomatopods may therefore be more marketable in the summer in the United States.

The GOM shrimp trawl fishery has an estimated 20,000 licensed boats and generates more than $500 million annually just by focusing on three species of penaeid shrimps: brown shrimp (*Farfantepenaeus aztecus*), white shrimp (*Litopenaeus setiferus*), and pink shrimp (*Farfantepenaeus duorarum* Burkenroad 1939) (Diamond 2004). Harvesting stomatopods by trawling in conjunction with other fisheries is likely to be the only method of utilizing them because aquaculture is not feasible for several reasons. First, *Squilla* may be carriers of deadly viruses (Chakraborty et al. 2002), which would prevent developing a multi-species aquaculture system with *Squilla*. Life history characteristics such as larval development stages requiring fluctuating diets and having high mortality rates after hatching (Rudloe 1971, Wortham-Neal 2002b), as well as adult cannibalism of *Squilla*, are additional limitations to aquaculture development.

In summary, *S. chydaea* and *S. empusa* are abundant in the GOM and are collected together roughly half the time. *Squilla chydaea* was collected in higher abundance but *S. empusa* was found in more trawls and had larger total biomass and larger individual biomass than *S. chydaea*. *Squilla empusa* reaches larger sizes than *S. chydaea*; however, because greater abundance of *S. chydaea* was collected, *S. chydaea* had a higher maximum catch rate year round/trawl. *Squilla chydaea* and *S. empusa* live at different depths but trawling year-round in high salinity waters at depths <60 m with maximum catch rates around 20 m would likely yield large biomass of stomatopods. *Squilla chydaea* and *S. empusa* are similar to *S. mantis* in their biology and behaviors and thus many aspects of the fishery for *S. mantis* could likely be used in the GOM. Mantis shrimp are currently harvested in abundance as bycatch in the penaeid fishery, and harvesting both penaeid shrimp and mantis shrimp at the same time might yield optimal economic and ecological efficiency.

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The Influence of Habitat and Fishing on Reef Fish Assemblages in Cuba

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THE INFLUENCE OF HABITAT AND FISHING ON REEF FISH ASSEMBLAGES IN CUBA

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ABSTRACT: The abundance of selected fish species was estimated using the stationary visual census technique in the northwestern region of the Cuban shelf. A total of 26,809 individuals of 32 species were counted in 1,172 stationary point censuses made at 10 reef sites along the coast. We found that the abundance patterns were most probably the consequence of the presence/absence of mangroves and seagrass beds in adjacent lagoon areas. A second factor influencing the spatial variation appeared to be overfishing on an east-west gradient, with lower abundances of commercially targeted species near Havana City in the east.

RESUMEN: La abundancia de especies de peces seleccionadas fue estimada usando una técnica de censo visual estacionario en la región noroccidental de la plataforma cubana. Se realizaron 1,172 censos puntuales estacionarios en 10 sitios arrecifales a lo largo de la costa. Se contaron en total 26,809 individuos pertenecientes a 32 especies. Se encontró que los patrones de distribución de abundancia son muy probablemente la consecuencia de la presencia o no de manglares y pastizales marinos en las áreas lagunares adyacentes. Un segundo factor que influye en la variación espacial parece ser un gradiente de sobrepesca en la dirección este-oeste, con abundancias menores de peces comerciales cerca de la Ciudad de La Habana, en el este.

INTRODUCTION

The function of mangroves and seagrass beds as nursery areas for coral reef fishes is well established (Heck et al. 2003, Mumby et al. 2004, Adams et al. 2006). The processes and mechanisms of connectivity from the back reef, e.g., mangroves and lagoons, across seagrass beds to the fore reef have been reviewed by several authors (Beck et al. 2003, Hughes et al. 2005, Sale et al. 2005, Cowen et al. 2006). At the species level, it is clear that there are more questions than answers about the function of backreef zones for coral reef fishes. Some species appear to be strongly dependent on seagrass and mangrove habitats (Nagelkerken et al. 2002, Dorenbosch et al. 2004), while other findings caution against a generalized hypothesis that back reefs are nursery habitats (Chittaro et al. 2005, Dorenbosch et al. 2007). There is some debate whether back-reef habitats significantly contribute to the fish population of the coral reef or only function as additional habitats (Beck et al. 2003, Heck et al. 2003).

One challenge is our inability to clearly define nursery habitats for coral reef fishes (Dahlgren et al. 2006, Sheaves et al. 2006, Layman et al. 2006). Based on visual census data in different habitats, Nagelkerken et al. (2002) suggested just 4 species heavily dependent on lagoons as nurseries. Seven additional species used the lagoon, but there was insufficient evidence to classify the lagoon as a nursery area. Dorenbosch et al. (2004) suggested that some species are highly dependent on the presence of bays with seagrass beds and mangroves as nurseries at the scale of whole islands. Mangrove habitats can be obligate nursery areas for the rainbow parrot fish, *Scarus guacamaia*, (Dorenbosch et al. 2006), and adult densities can be significantly greater at reefs with adjacent mangroves (Mumby et al. 2004).

In contrast, Chittaro et al. (2005) found that only 4 of the 6 most abundant and commercially important species (*Haemulon flavolineatum*, *H. sciurus*, *Lutjanus apodus* and *L. mahogoni*) showed higher numbers of juvenile fish in mangrove and/or seagrass habitats with adjacent coral reefs, and at just 4 of 9 sites studied. Dorenbosch et al. (2007) found that most fish species using seagrass and mangroves as juvenile habitats were absent from, or showed reduced densities on adjacent, but distant coral reefs (> 9 km away). They proposed that seagrass and mangrove areas should not be generalized as juvenile habitats because habitat configuration, e.g., distance between, may limit connectivity between mangroves, seagrass beds, and coral reefs.

In a recent review, Adams et al. (2006) classified coral reef fishes based upon their inter-habitat, ontogenic migration patterns. The authors define Group A as habitat specialists using the same habitat at all life stages, Group B as habitat generalists which are not site-attached and use a variety of habitats, and Group C as ontogenetic shifters. The latter species switch habitats during their life, such as the transition from settlement to juvenile to maturing adults. Habitat connectivity from back to fore reef is predicted to be critical for such species. Results by Gratwicke et al. (2006) showed that a detailed review of the natural life-history strat-

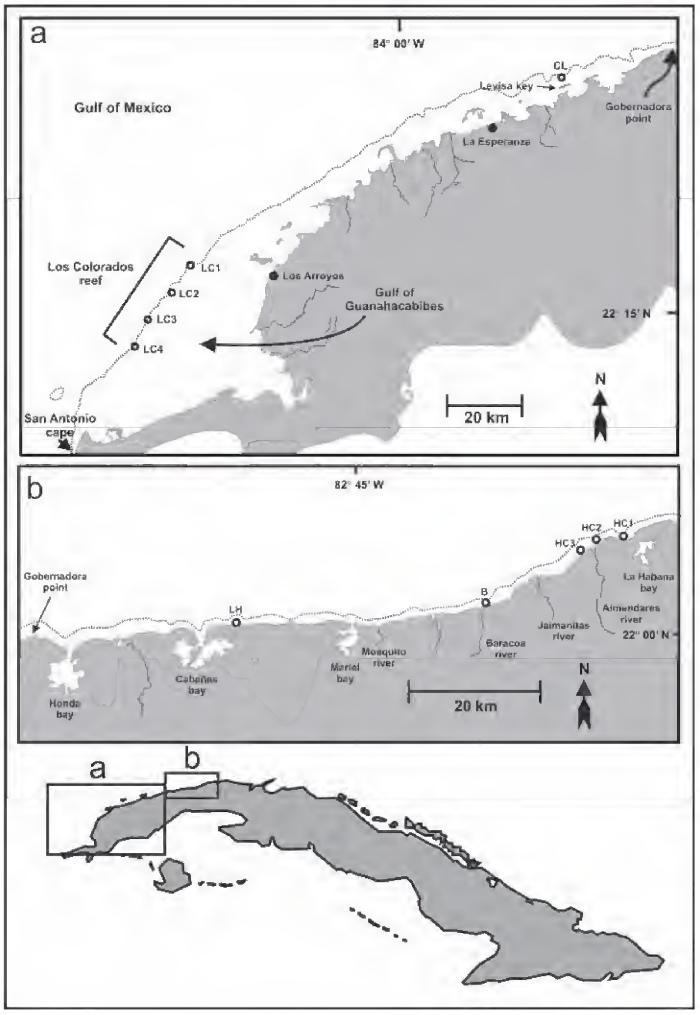

Figure 1. *Northwestern region of Cuban shelf. a. Western portion; b. Eastern portion. Open circles indicate studied reef areas.*

egies and habitat requirements are required before making further generalizations about the role of nearshore habitat types as nurseries for reef fishes.

From 1996 to 2006, the fish assemblages and habitats of northwestern Cuba have been investigated (Aguilar et al. 1997, Gonzalez-Sanson et al. 1997, Aguilar et al. 2004, Guardia et al. 2005). We re-examined these data in relation to the potential habitat connectivity within reef complexes along the coast, and discuss how species complexes are organized by the degree of potential connectivity. In addition, a pollution gradient along this coast (Aguilar et al. 2007), in conjunction with probable overfishing, may influence fish assemblages. These latter factors are incorporated into our observations and discussion.

Materials and methods

Study Area

The study was conducted in the northwestern region of the Cuban shelf. The main shallow-water habitats in this region are fringing coral reefs, seagrass beds and nearshore mangrove prop-root muddy environments. The estimated total surface area of this shelf region, which extends from the high tide line to the 200 m isobath, is 4,050 km^2. Mean shelf depth is 4-5 m, although at some locations in the Gulf of Guanahacabibes the depth can be up to 18 m (Figure 1). Reefs included in the study are defined in Table 1.

At Havana City in the east, a frontal fringing reef develops mainly in the 12-15 m deep terrace, at 200-300 m offshore. The area between the shore and the reef is an almost bare rocky plain. Since the impact of pollution coming from the city varies notably along the coast from Havana harbor entrance (most polluted area) towards the southwest, the reef was divided in three different sites named HC1, HC2 and HC3, and analyzed separately. More details and the rationale for this division can be found in Aguilar et al. (2004) and Aguilar et al. (2007).

Baracoa (B) is a fringing reef near a small town of the same name. The reef has a well developed crest (length = 1 km) dominated by *Acropora palmata* and a small seagrass bed (< 4 ha) in the lagoon. The shoreline is highly modified by man-made structures and no mangrove growth is present. A heavily polluted small coastal lagoon (ca. 20 ha) near this reef has a small mangrove growth (< 1 ha). La Herradura (LH) is a fringing reef growing on the west side of a cove of the same name. It has a poorly developed crest dominated by *A. palmata* and a well developed, but small seagrass bed in the lagoon (< 2 ha). The shore is a sandy beach with no mangroves. Cayo Levisa (CL) is the reef of the key with the same name. It has a poorly structured crest dominated by *A. palmata* and a well-developed seagrass bed in the backreef zone. Mangroves are very abundant in the southern shore of the key and along the mainland. The Los Colorados reef (LC1 to LC4) is a large bank-barrier reef (length ~ 40 km with a wide crest area of *A. palmata*). Significant expanses of seagrass beds in the lagoon (more than 500 km^2) abut well developed mangroves along the shore (length of coast with mangrove ~ 80 km).

Levels of pollution and fishing pressure were defined for each reef (Table 1). Two rank scales were prepared based on anecdotal information, geographic position of the reefs in relation to main pollution sources, and distance to urban centers. These scales are defined as follows:

Pollution:

1. Very low. Reefs which are very far (> 30 km) from any urban center or land-based pollution source. No evidence of any contamination.
2. Low. Reefs which are far from any urban center or industrial waste source, but not far from the coast. Some pollution from agriculture is assumed in this case.
3. High. Reefs which are near the coast in narrow shelf areas adjacent to big urban centers but without the direct impact of big discharges.
4. Very high. Reefs which are near the coast in narrow shelf areas adjacent to big urban centers and are receiving the

TABLE 1. *Geographical position, associated lagoonal habitats, sampling dates and sizes, pollution levels and fishing impact of the studied reefs (see also Figure 1 and text for details).*

Reef	Reference coordinates	Lagoon habitats	Sampling dates	Number of censuses	Pollution [A] level	Fishing [B] impact
HC1	23° 08.549′ N 82° 22.012′ W	Rocky plain	Feb-Mar 2000	96	4	3
			Jun 2000	96		
HC2	23° 08.250′ N 82° 24.565′ W	Rocky plain	Feb-Mar 2000	64	3	3
			Jun 2000	64		
HC3	23° 07.359′ N 82° 26.087′ W	Rocky plain	Feb-Mar 2000	80	3	3
			Jun 2000	80		
B	23° 03.362′ N 82° 335.97′ W	Seagrass bed	April 2004	99	2	2
			September 2004	99		
LH	23° 01.481′ N 82° 55.014′ W	Seagrass bed	March 1996	108	2	2
			October 1996	109		
CL	22° 52.890′ N 83° 34.093′ W	Seagrass bed & mangrove	June 2003	27	2	2
			October 2003	70		
LC1	22° 23.023′ N 84° 36.589′ W	Seagrass bed & mangrove	March 2006	45	1	1
LC2	22° 18.320′ N 84° 40.235′ W	Seagrass bed & mangrove	March 2006	45	1	1
LC3	22° 13.970′ N 84° 44.091′ W	Seagrass bed & mangrove	March 2006	45	1	1
LC4	22° 09.451′ N 84° 46.373′ W	Seagrass bed & mangrove	March 2006	45	1	1

A: 1–very low; 2–low; 3–high; 4–very high. B: 1–high; 2–very high; 3–exceedingly high.

direct impact of big pollution discharges (e.g. tidal discharge from a heavily polluted port).

Fishing pressure:

1. High. Reefs which are very far (> 30 km) from any urban center. Only commercial vessels fish in these reef areas and target species are big-sized species (e.g., larger snappers, groupers, jacks).
2. Very high. Reefs which are far from any urban center but near small coastal villages. Almost no commercial fishing and heavy subsistence fishing pressure mostly with small boats.
3. Exceedingly high. Reefs which are near the coast in narrow shelf areas adjacent to big urban centers. A very high subsistence fishing effort by people using small rafts, boats, spearguns, gill nets with small mesh size and traps.

Sampling Procedures

In all reefs but Los Colorados, sampling occurred on two different dates. In these cases data of each sampling date were treated as separate units in our analyses (Table 1).

The abundance of fish was estimated using the stationary visual census technique of Bohnsack and Bannerot (1986) with some minor modifications. The nominal radius of the observing cylinder was 5 m. As the fish assemblage composition can vary substantially between different biotopes within a reef (crest, spur & grooves, terrace, etc.), we made repeated censuses in each main biotope at each reef. Counts

TABLE 2. *Mean, minimum and maximum abundance estimations, frequency of occurrence (F) in the 16 samples and total length (TL) range of fish counted for each species included in the study. Rank-correlation values (r_s) were calculated between species abundance at each site and rank of sampling sites along the coast from west to east. Probabilities (p) for r_s values which are significant are in bold.*

Species	Individuals per count			F	TL (cm)	r_s	p
	Mean	Min	Max				
Cephalopolis cruentata	0.15	0.00	1.37	11	14 - 20	0.24	0.365
Cephalopolis fulva	0.47	0.02	2.16	16	10 - 25	0.64	**0.007**
Epinephelus ascencionis	0.02	0.00	0.13	5	20 - 30	0.58	**0.018**
Epinephelus guttatus	0.13	0.00	1.31	12	15 - 40	0.64	**0.007**
Epinephelus striatus	0.02	0.00	0.11	6	30 - 60	0.80	< **0.001**
Lutjanus analis	0.05	0.00	0.27	10	40 - 75	0.29	0.273
Lutjanus apodus	0.35	0.00	1.00	11	15 - 40	0.61	**0.012**
Lutjanus cyanopterus	0.02	0.00	0.29	5	40 - 60	0.76	**0.001**
Lutjanus synagris	0.29	0.00	1.15	10	15 - 25	-0.83	< **0.001**
Ocyurus chrysurus	0.46	0.00	1.73	14	15 - 30	0.14	0.616
Gerres cinereus	0.05	0.00	0.15	9	15 - 20	-0.57	**0.020**
Haemulon aurolineatum	0.28	0.00	3.59	6	10 - 15	-0.27	0.315
Haemulon carbonarium	0.10	0.00	0.86	7	10 - 15	-0.76	**0.001**
Haemulon chrysargyreum	0.30	0.00	1.60	9	10 - 15	0.17	0.522
Haemulon flavolineatum	2.27	0.30	5.54	16	10 - 20	-0.44	0.084
Haemulon plumieri	1.01	0.08	3.16	16	10 - 25	0.29	0.272
Haemulon sciurus	0.46	0.04	1.19	16	10 - 25	0.39	0.134
Chaetodon capistratus	1.00	0.20	2.07	16	8 - 17	-0.32	0.224
Chaetodon ocellatus	0.22	0.00	0.45	15	12 - 18	-0.69	**0.003**
Chaetodon sedentarius	0.15	0.00	0.70	11	10 - 15	-0.73	**0.001**
Chaetodon striatus	0.45	0.07	2.18	16	8 - 15	0.20	0.459
Lachnolaimus maximus	0.03	0.00	0.24	5	25 - 40	0.70	**0.002**
S. iseri /taeniopterus	2.92	0.18	15.43	16	5 - 30	0.85	< **0.001**
Sparisoma atomarium	0.19	0.00	2.09	7	3 - 12	0.87	< **0.001**
Sparisoma aurofrenatum	1.17	0.02	5.01	16	10 - 30	0.14	0.612
Sparisoma chrysopterum	0.24	0.00	0.97	14	10 - 30	0.01	0.965
Sparisoma rubripinne	0.39	0.00	2.03	15	15 - 30	0.16	0.548
Sparisoma viride	1.11	0.09	4.49	16	15 - 30	0.86	< **0.001**
Acanthurus bahianus	5.01	0.96	9.68	16	10 - 25	-0.60	**0.014**
Acanthurus chirurgus	0.62	0.00	1.94	14	13 - 25	-0.76	**0.001**
Acanthurus coeruleus	2.90	0.93	7.55	16	10 - 25	0.34	0.192
Sphyraena barracuda	0.07	0.00	0.44	8	65 - 120	0.83	< **0.001**

per biotope were pooled for each reef (Table 1). Data are given as mean number of individuals per census.

Original data included all species observed. For the present analyses, not all species or groups of species (families and/or genera) were selected. We excluded Adams et al.'s (2006) habitat specialists and generalists (Groups A and B, e.g. damselfishes and small wrasses, respectively). Nocturnal species were also excluded as they are highly cryptic during the day. Species included were the families Acanthuridae, Scaridae, Lutjanidae, Serranidae (genera *Epinephelus* and *Mycteroperca*) and Chaetodontidae. Three additional species which have been considered habitat-shifters were also included: *Sphyraena barracuda*, *Lachnolaimus maximus* and *Gerres cinereus*. The species *Scarus iseri* and *S. taeniopterus* were usually indistinguishable in the field and we hereafter refer to them as *S. iseri/taeniopterus*.

Statistical Analysis

Hierarchical agglomerative cluster analyses were performed using as dissimilarity measures the Bray-Curtis distance on fourth-root transformed counts for samples grouping and 1-r_s, (r_s = Spearman's rank correlation coefficient) for inverse analysis (clustering species; Boesch 1977). In all cases the UPGMA clustering algorithm was used. Non parametric multidimensional scaling (MDS) was employed for ordination of samples based in same distance matrices as cluster analyses. The combination of clustering and ordination analysis has been described by Clarke and Warwick (2001) as the most effective way to check the adequacy and mutual consistency of both representations. One-way ANOSIM (Clarke and Warwick 2001) was used to verify the significance in fish assemblage composition of samples classified *a priori* by the presence/absence of seagrass and/or mangrove. All analyses were made using PRIMER 5.5 and STATISTICA 6.0 software.

As a complement to Cluster and ANOSIM analyses, rank correlation coefficients were calculated between the abun-

dance of each species and the ranks of sites according to their position along the coast (rank 1 for HC1 to rank 10 for LC4 - see Table 1). In this analysis, species more abundant towards the east will have significant negative coefficient values and those more abundant towards the west will have significant positive coefficient values.

Results

A total of 26,809 individuals of 32 species of the selected groups were counted in 1,172 stationary point censuses (Table 2). The most abundant species were the medium-sized herbivores *A. bahianus*, *A. coeruleus*, *S. iseri/taeniopterus* complex, *S. aurofrenatum* and *S. viride*, and the medium sized small-invertebrates feeders *H. flavolineatum* and *H. plumieri*. Only two species of higher trophic levels were abundant enough to be included: *E. striatus* and *S. barracuda*.

All the species of *Mycteroperca* spp. (large groupers) were extremely scarce and were not included in further analyses. Larger herbivores were also rare; two individuals of *Scarus coelestinus* were observed and no *S. coeruleus* or *Scarus guacamaia* were observed.

After the numerical classification and multidimensional scaling, samples formed three well separated groups (Figure 2). Group A included all the sites off Havana City. Group B included samples at Baracoa and La Herradura reefs. Group C was in the Levisa key and Los Colorados reefs. Groups correlated well with the presence or absence of seagrass beds and/or mangroves in the lagoon, the fishing pressure levels and pollution levels (Table 1).

ANOSIM based on presence/absence of seagrass or mangrove produced a global test that was significant ($R = 0.962$, $p = 0.001$). The pairwise comparisons were also significant ($R_{A,B} = 0.960$, $p = 0.005$; $R_{A,C} = 0.981$, $p = 0.002$; $R_{B,C} = 0.960$, $p = 0.005$). This pre-determined classification coincides with the results of hierarchical classification and ordination methods.

Two well differentiated groups of species were obtained (Figure 3). The first group included all species of the genus *Acanthurus*, the majority of species in genera *Haemulon* and *Chaetodon*, *G. cinereus* and one species each of Lutjanidae and Scaridae. The second group includes all species in genus *Epinephelus*, the majority of species in the families Lutjanidae and Scaridae, *S. barracuda*, *L. maximus* and just two species of the genus *Haemulon* and one of the genus *Chaetodon*.

Of the 32 species included in the study, 18 showed significant correlation with the ordering of sites (Table 2). Some correlations were negative, indicating increase of abundance towards the east. This was the case for *A. bahianus*, *A. chirurgus*, *C. ocellatus*, *C. sedentarius*, *G. cinereus*, *H. carbonarium* and *L. synagris*. Other correlations were positive indicating increase of abundance towards the west. This was the case for *E. ascencionis*, *E. guttatus*, *E. striatus*, *L. apodus*, *L. cyanopterus*, *S. iseri/taeniopterus*, *S. atomarium* and *S. barracuda*.

A joint plot of abundances for species which showed significant correlation revealed a clear pattern (Figure 4) that explained site clustering (Figure 2): Group A is dominated by species which are more abundant in the east; Group C includes mainly species most abundant in the west; and Group B is defined by species which have similar abundances across the entire coast. A more detailed comparative analysis by species gives a better understanding of the patterns observed.

The surgeonfishes, *A. bahianus* and *A. chirurgus*, showed a high correlation in their abundances ($r_s = 0.804$, $p = 0.0002$) while both had a weak correlation with *A. coeruleus*, i.e., the first two species were more abundant towards the east, while *A. coeruleus* showed no significant trend in its abundance. Similarly, *C. ocellatus* and *C. sedentarius* were highly correlated ($r_s = 0.931$, $p < 0.0001$). These species were significantly more abundant toward the east and had no significant correlation with other species in the genus. The abundance of two grunts, *H. flavolineatum* and *H. chrysargireum* behaved in the same fashion, as they were highly correlated ($r_s =$

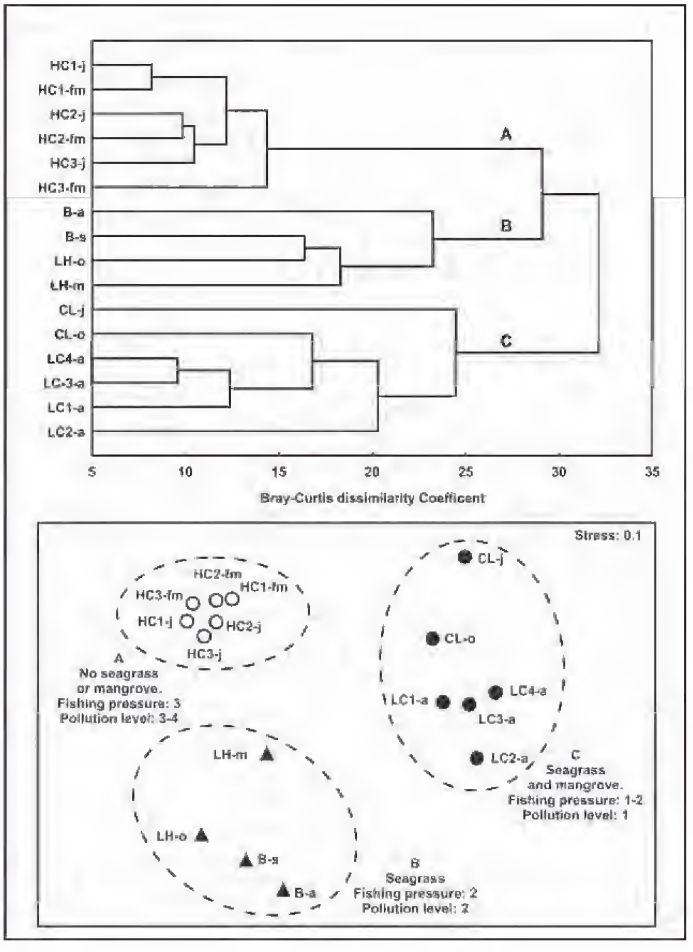

Figure 2. *Classification (upper panel) and MDS ordination (bottom panel) of samples. Each sample is identified by the acronym of the sampling reef area (capital letters and numbers) and the date (month) of sampling (lowercase letters). See Table 1 and Figure 1 for more details.*

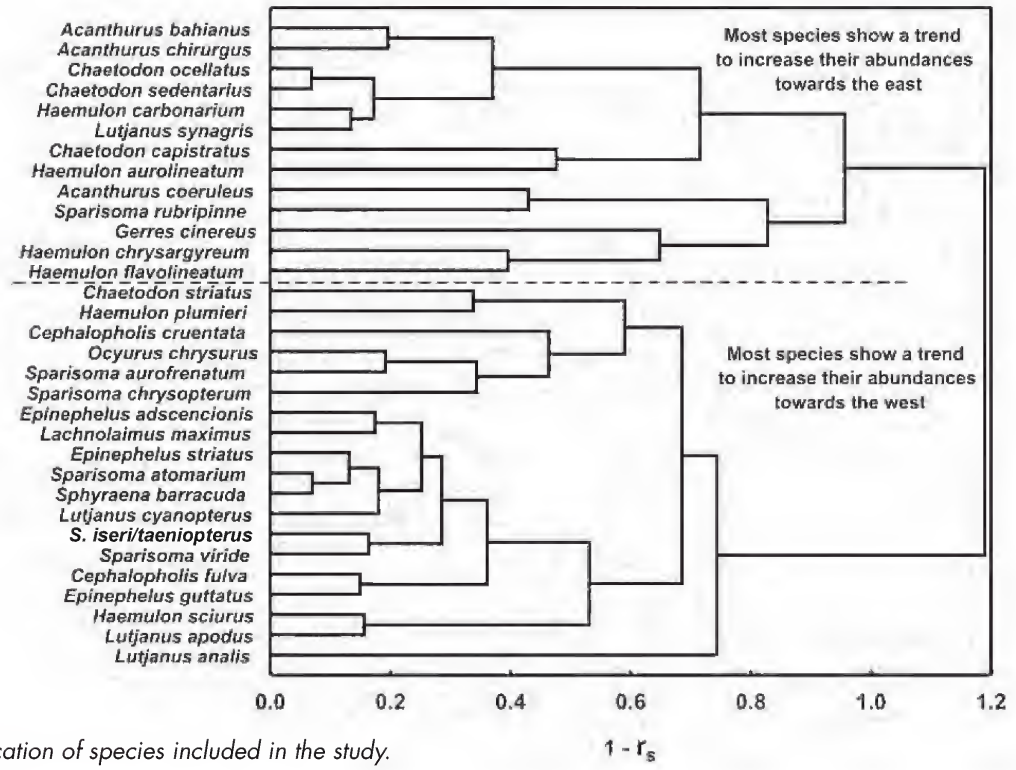

Figure 3. *Classification of species included in the study.*

0.604, p = 0.013) and showed no significant correlation with other species in the genus. They also significantly increased in abundance toward the east.

The abundances of small groupers (*Epinephelus* spp. and *Cephalopholis* spp.) were, in general, significantly correlated. All species but *C. cruentata* showed high positive correlation with site ranks, were more abundant towards the west, and were closely clustered (Figure 3). The same was true for the majority of parrotfishes (Scaridae) with the notable exception of *S. rubripinne* which was negatively correlated with other species of the family. The species complex *S. iseri/taeniopterus*, *S. atomarium* and *S. viride* were significantly correlated, and these species were also significantly correlated with site ranking, increasing in abundance toward the west.

Discussion

In many cases, we found that patterns of fish abundance followed expectations based upon habitat distributions, especially distribution of mangroves and seagrass. To this extent, our data support the hypothesis that the presence of appropriate nursery areas near the reefs enhances the abundance of species depending on these nursery areas. However, we also believe that fishing and pollution modified many fish abundance patterns, confounding some of the fish-habitat associations. For example, fishing pressure and pollution levels increased from west to east, whereas lagoonal habitat complexity increased from east to west. This was most evident near Havana City, which has experienced severe overfishing.

The use of mangroves, seagrass beds and/or algal growth in shallow, near-shore waters has been reported for juveniles *E. striatus* (Dahlgren and Eggleston 2001), *L. apodus* and *S. iseri* (Nagelkerken et al. 2002, Chittaro 2005), *L. cyanopterus* (Heyman et al. 2005), and *S. barracuda* (Nagelkerken et al. 2002). These species showed a significant increase of their abundances in reefs with adjacent seagrass and mangrove habitats that increased in occurrence in western areas of the northern Cuban coast. The mangrove and seagrass beds are very scarce towards the east and near Havana City, but there are always small areas with limited nursery areas for these species, e.g., estuarine mangrove at river mouths and small embayments. Some of these species were present or more abundant near Havana City in the past, but they are targeted and thus have been decimated by overfishing (Aguilar 2005). Grober-Dunsmore et al. (2007) found a very weak relationship of Epinepheline fishes with the associated seagrass surface, but recognized that this was an unexpected result because the current view holds that this group is highly dependent on areas of seagrass. In our case, Epinepheline fishes increased in abundance as habitat complexity within the reef system increased, i.e., in the west.

Cocheret de la Moriniere et al. (2002) found that juvenile *A. bahianus* were present mostly in shallow reefs and seagrass areas near these reefs. This species, which settles mostly in shallow waters of the reef and is less dependent on seagrass and mangroves to complete its life cycle, was most abundant in the less complex habitats (eastern area) along the northwestern Cuban coast. In addition, the species may gain some competitive advantage where other important herbivores such as parrotfishes are less abundant.

Parrotfishes are more dependent on the presence of seagrass adjacent to the reef (Dorenbosch et al. 2004, Mumby 2004) and this is particularly true for the most abundant species in the present study, *S. iseri/taeniopterus* (Adams and Ebersole 2002, Cocheret de la Moriniere et al. 2002, Nagelkerken et al. 2002). The pattern showed by this species in our study, with abundance increasing significantly towards the west where there was more seagrass and/or mangroves, agrees with previous research.

We did not predict that *L. synagris* would increase in abundance significantly towards the east. However, *L. synagris* is one of the most important species in the commercial fishery, which takes place in the broad shelf area forming the backreef of Los Colorados reef at the western portion of our study area. A possible explanation is the species' use of diurnal shelter sites that are mostly patch reefs common in the seagrass beds away from the forereef. Along the eastern portion of our study area, the shelf is narrow with poorly developed or no back reef areas. We believe the species is apparently more abundant in the east because it has no alternative habitat to the forereef and there is less fishing pressure for this species, i.e., spear gun vs commercial fishing by net in the east and west, respectively.

We believe that abundances of large sized species in this study have been affected by high fishing pressure on targeted species (e.g. Aguilar et al. 1997, Aguilar et al. 2004). Aguilar (2005) interviewed local fishermen from Havana City, and they reported the number of species that reach large sizes and

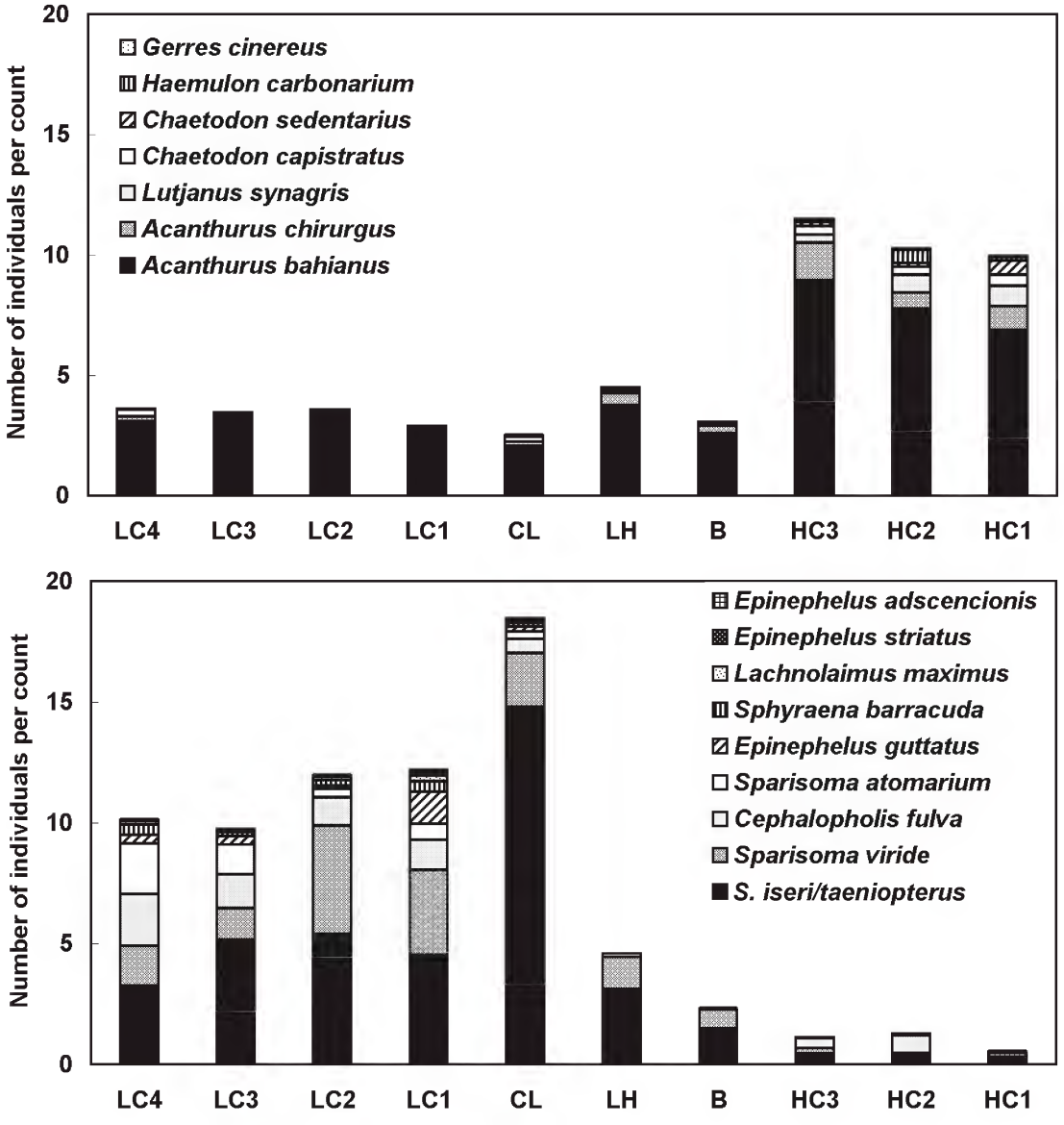

Figure 4. *Abundance estimates for species which showed a significant increase in abundance towards the east (upper panel) and towards the west (bottom panels). Species included are those which had significant rank correlations with sites (see Table 2).*

were captured frequently in the 1970s have almost entirely disappeared, e.g., *S. guacamaia*, *S. coeruleus*, *S. coelestinus*, *L. maximus*, *Mycteroperca bonaci*, *Epinephelus itajara*,and *Lutjanus jocu*. Only the mutton snapper, *Lutjanus analis*, appears with some abundance during the reproduction time ("runs") and as an effect of some meteorological events (e.g. cold fronts, hurricanes) that cause "arribazones" (fish coming near the shore). Fishermen reported a significant decrease over time in the mean size of individuals observed or captured (e.g., Hutchings 2005). A similar change in fish assemblage composition was reported by Beets et al. (2003) for the Virgin Islands. There, the biomass of large predators appears to be reduced and biomass of herbivores and invertebrate feeders proportionally increased as fishing intensity and other human disturbances increased.

Although there is no formal study on the topic, we assume that there has been a sustained increase in subsistence fishing in the area near Havana City, as a consequence of the economic crisis which started at the beginning of the 1990s. Increasing spear-gun fishing is the main cause of the observed changes into the 1980s, thereafter illicit fishing began with gillnets adding pressure on small and medium size species such as parrotfishes, surgeonfishes and grunts.

Based on our observations reported herein and additional interviews with commercial fishers and tourist fishing operators, increases in fishing pressure are expanding along the entire northwestern coast to the area of Cayo Levisa. Farther west, the human presence is much less and is concentrated in small fishing villages which use mostly commercial fishing gears to target the largest species. In general terms, the main impact of fishing in the entire northwestern Cuban shelf is the reduction of larger fish abundance with the consequence of highly modified fish assemblages along the coast (Aguilar et al. 2004).

Increasing human incursions into coastal ecosystems of the Caribbean most probably intensify the negative impacts on reef fishes. Mumby et al. (2004) report that the parrotfish *S. guacamaia* underwent local extinction during the past 30 years at Glovers Reef, Belize. These authors consider that the extinction of this species at Glovers Reef was most probably due to the removal of its nursery habitat and overfishing. Historical overfishing and mangrove deforestation will certainly work synergistically to reduce herbivory and secondary production at Caribbean coral reef ecosystems (Beets et al. 2003). In our study, the most abundant species were medium-sized herbivores. The removal of large predators and competitors (larger herbivores) by fishermen could allow an increase in the abundance of smaller bodied fishes by competitive release processes.

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SEAGRASS LOSS IN BELIZE: STUDIES OF TURTLEGRASS (*THALASSIA TESTUDINUM*) HABITAT USING REMOTE SENSING AND GROUND-TRUTH DATA

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ABSTRACT: Spatial and temporal change in turtlegrass (*Thalassia testudinum*) habitat of the South Water Caye Marine Reserve (SWCMR) in Belize were analyzed using satellite images backed up with ground-truth data. We had two primary objectives. First, we wanted to determine areal expanse of seagrass across a large area (~12 km by 3 km) of the SWCMR, and address its change over time. We used paired satellite images taken during 2001 and 2005 to determine coverage by seagrass and measure temporal variables. These analyses recorded an overall seagrass loss of 1.8% (52.3 ha) during the 4 yr period. Secondly, we wanted to determine whether seagrass gains or losses were consistent across the study area. Replicate sampling was used as a statistical basis and confirmed a significant loss of seagrass across the region. It also helped identify two regions of significant seagrass loss; one 600 ha area lost 12.4% of its seagrass; another 240 ha area lost nearly 40%. These components helped us assess seagrass habitat in an area perceived as critical to Belize fisheries, and provided the scale and statistical rigor necessary to adequately assess a broad region of study. The salient results from our study were not the magnitude of seagrass loss *per se*, but the loss in seagrass habitat from an area that is thought to be relatively pristine. Seagrass-habitat loss in this region of the Caribbean Sea may be evidence that even near-pristine areas can be impacted by anthropogenic factors. Determining the causes of habitat loss may help prevent loss of productivity, habitat, and livelihood for the associated human and nonhuman communities.

INTRODUCTION

Seagrass ecosystems are among the most productive on earth, and their ecological and economic importance is becoming obvious as they diminish worldwide. Seagrass habitats are vital primary producers that improve water quality, promote sedimentation, recycle nutrients, and provide structure that serves as refuge and nursery ground for fisheries species (e.g., Moriarty and O'Donohue 1994, Hall et al. 1999, Short and Wyllie-Echeverria 2000, Gillanders et al. 2003, Green and Short 2003, Corlett and Jones 2005). The interest in seagrass is global, as is the research effort to assess changes in seagrass habitat. Many studies centered around areas of long-term loss of seagrass (e.g., Short and Short 2003, Duarte et al. 2008), while others documented regions of seagrass recovery (e.g., Virnstein et al. 2007).

Turtlegrass (*Thalassia testudinum*) is a common seagrass species in waters of the tropical western Atlantic from Venezuela to eastern Florida and the Bahamas (den Hartog 1970), and is one of many species adversely affected by natural and anthropogenic factors. Researchers contend that protecting seagrass habitat may prevent loss of commercial fisheries, improve water quality, and help maintain healthy interrelated communities (Ward 1998).

The purpose of this study was to discern the stability of the turtlegrass-dominated seagrass community in the South Water Caye Marine Reserve (SWCMR) of Belize by using satellite imagery. This reserve is generally perceived to be pristine, far from the coastal influences that adversely affect most other seagrass communities, and an area of interest to Belize fisheries and tourism. We had two primary objectives. First, we wanted to determine areal expanse of seagrass, and address its change over time by analyzing satellite images taken 4 years apart. Secondly, we wanted to determine whether seagrass gains or losses were consistent across the study area, and used ground truthing to confirm our observations by visiting sites of concern identified by satellite images. The multiple components of our study helped assess seagrass habitat in an area perceived as critical to Belize fisheries, and provided the scale and statistical rigor necessary to adequately assess a broad region of study.

METHODS

Study Area

The study area was in the South Water Caye Marine Reserve, Belize (Central America), along Belize's Caribbean coast, about 14 km from the mainland. It is part of the Belize barrier reef ecosystem, which was designated as a UNESCO (United Nations Educational, Scientific, and Cultural Organization) World Heritage Site in 1996. The biological communities, physical oceanography, geology, and history of the area were summarized by Rützler and Macintyre (1982).

The area of habitat classification for this study (16° 54' to 16° 46' N; 88° 04' to 88° 07' W) was a ~3 km wide area

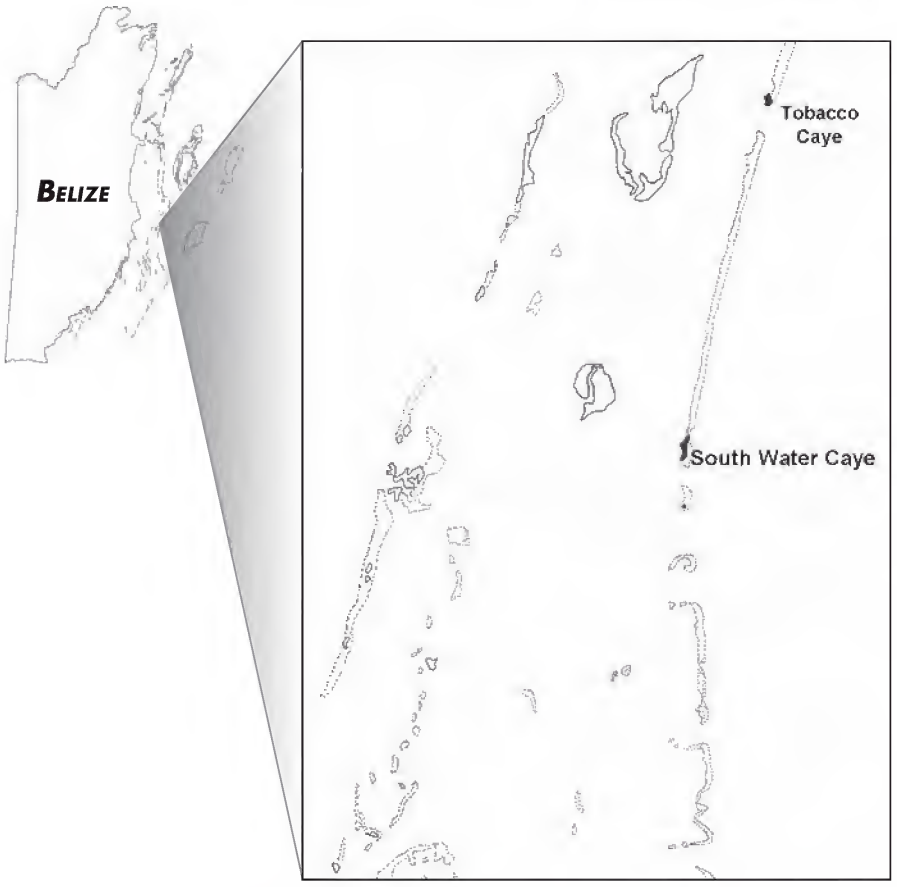

Figure 1. *Map of the study area in Belize with details of the islands (solid lines) and reefs (dotted lines) in the South Water Caye Marine Reserve (16° 54′ to 16° 46′ N; 88° 04′ to 88° 07′ W). Two major channels open into the lagoon. Tobacco Channel is just south of Tobacco Caye (shaded black); South Water Channel is just south of South Water Caye (shaded black).*

from Tobacco Caye southward beyond Curlew Caye (12 km), extending from the western-most region of back reef (2.5 m depth) to the lagoon (3-7 m depth) (Figure 1). We used a region of similar depth so we could attain consistent spectral contrast for classifying the seagrass (as per Andréfouët et al. 2003). There were no strong tropical cyclones through the area during this study period that destroyed or buried seagrass, nor did our divers observe any effects from previous storms, such as they observed elsewhere in the Caribbean.

Satellite Images

We used paired satellite images to determine change in areal coverage of the region by seagrass. In order to assess both total change in habitat and determine the region most affected by change, we analyzed seagrass habitats of the study area on two scales of distribution. Our broad-scale analyses included a large region of the SWCMR as an entity. Our medium-scale analyses included replicate samples (4 ha each) to address questions on a smaller scale (tens of meters). The replicates provided a means of statistical assessment of habitat change.

The images used in this study were acquired by the University of Mississippi Geoinformatics Center (Oxford, Mississippi), and consisted of an IKONOS image of 12 September 2001, and a Quickbird image taken on 10 April 2005. Both images covered the areas of study with minimal cloud cover (< 0.1% in the IKONOS image; 4% in the Quickbird image). As necessitated by differences in cloud cover, a masking technique was used to render the images comparable (see below). The two images were of high-spatial resolution (IKONOS = 1 m; Quickbird = 0.61 m) and consisted of 4 spectral bands of the same wavelengths; these similarities allowed for maximum analysis and comparison. The band widths for both images were 0.45-0.90 µm for the panchromatic band, 0.45-0.52 µm for band 1, 0.52-0.60 µm for band 2, 0.63-0.69 µm for band 3, and 0.76-0.90 µm for band 4. The QuickBird image had a significant presence of sun-glint reflection due to the angle of the sun (time of day) when the scene was collected. A process was used to remove the sun glint from the QuickBird image (see below).

Several pre-processing techniques were necessary to begin classifying and analyzing the satellite images. First, the panchromatic bands and the multispectral bands were combined to produce the pan-sharpened, multispectral imagery. Image rectification was performed in order to geometrically correct both the 2001 and 2005 images. Ground control points of known location in all areas of the images were used to correct the images, with most of them being geo-referenced points (piers, homes, and other unaltered structures). Nearest-neighbor resampling was used instead of cubic convolution resampling due to the degradation of textural information which occurs using cubic-convolution resampling (Andréfouët et al. 2003).

Removal of the sun-glint from the 2005 QuickBird image was required before further pre-processing steps could be initiated. Confused-resolution images result when light is reflected off of the crests and slopes of waves in the image (Hochberg et al. 2003). This reflection is known as sun glint, and may cause significant problems when classifying the image. The sun-glint-affected pixels in the NIR band are also present in the visible bands. In order to correct for the glint, a method detailed by Hedley et al. (2005) was used on the 2005 image. The steps for removal of the glint were completed by using ITT's ENVI 4.5, and the linear regression calculations were completed in Microsoft Excel (Hogrefe et al. 2008).

Both the IKONOS and QuickBird images were corrected and enhanced using common remote-sensing techniques such as georectification, land/cloud masking and image resampling, in addition to the aforementioned glint removal. The islands (present in both images) and shallows were de-selected from the images prior to classification of seagrass. The clouds in the 2001 image were on the margin of the study area; they were de-selected before classification. The presence of clouds in the 2005 image required that a cloud mask (delineated from the 2005 image) be applied to both images. This was done in order to generate classified images that represent the same area processed during the unsupervised classification.

The final step of preprocessing prior to the unsupervised classification was resampling the 2005 QuickBird image, with a 0.61 m pixel resolution, to match the lower 1.0 m pixel resolution of the 2001 IKONOS image. After resampling, both images had a pixel resolution of 1.0 m, which allowed for the unsupervised classification to be performed. These standard post-processing steps were completed using ERDAS Imagine 9.1.

An unsupervised classification was performed using Leica Geosystem's ERDAS Imagine 9.1. The classifications divided the images' digital numbers into 60 classes. The classification ran through 35 iterations up to 99% convergence. After classification of the two images, the 60 classes of each image were merged into 5 classes for preliminary assessment of the images. These 5 classes included several categories of seagrass density. An accuracy assessment was performed in order to draw an estimate of overall accuracy of the classifications. The method requires an error matrix (confusion matrix) to determine the accuracy of the classifications, in which classifications from the satellite images are compared with ground-truth data (Congalton and Green 1999). The preliminary accuracy assessment yielded values at about 90%; but those were below values necessary to support statistical analyses based on seagrass density. For further analyses the 60 classes were merged into 2 classes: one contained pixel values of all densities of seagrass combined; the other class contained pixel values that represented lack of seagrass (sand). Now the ground-truth data matched the classified images with high accuracy. From those data we totaled the number of pixels of each habitat to compare differences between the two images.

Ground Truthing

A field campaign was designed to collect ground-truth data to assess accuracy and spatial resolution of the images. We mapped the region with swim transects during March, May, and July 2001, May and June 2002, June 2003, and May and December 2004. We conducted surveys with glass-bottomed buckets, and made SCUBA observations during May and June 2005 for further rapid visual assessment (Mumby and Harborne 1998). After the 2001 surveys, a preliminary benthic map of seagrass habitat, distinguished by seagrass-density categories, was created. The map was based on ground truthing, an unsupervised classification using the ISODATA algorithm, and a 2001 satellite image (see below). We selected 162 points that were deemed references to check benthic coverage and image accuracy, and increased this number to 500 points with subsequent ground-truth surveys. Observations at the 162 points included estimates of seagrass density (dense, > 30 shoots/0.25 m^2; moderate, 5-29 shoots/0.25 m^2; sparse, < 5 shoots/0.25 m^2). The data taken at the additional points included only seagrass presence or absence. Each point was geo-referenced using a Garmin GPS 12CX with a positional accuracy of ± 1 m. After surveying each of the initial 162 points selected, the habitats of sand and seagrass in the area immediately surrounding the point (tens of meters) were also surveyed and recorded.

Ground truthing during preliminary work helped ascertain the adequacy of the region for study, and determine the distribution of seagrass and adjacent ocean-bottom habitats. We used numerous habitat categories during preliminary ground-truth data collection, including several seagrass-density categories, bare sand, sand with sparse algae (and no seagrass), and mixed-species habitats (turtlegrass, other seagrass species, algae, coral, and sponges). Some shallow areas of the region (< 2 m) supported algal mats and coral-rubble habitat with minimal seagrass. We found that those shallow regions, especially the back-reef areas (behind the reef crest) and coral-rubble habitats surrounding islands, had seagrass that was mixed with algae (usually *Ulva* spp.) and other seagrass species that increased potential for error in our classifications. Algal mats resemble seagrass in remotely sensed data, and might have obfuscated the analyses. We eliminated those shallow-water habitats, patch reefs, and recently dredged areas near South Water Caye from further classification. This preliminary work provided a study area (~ 12 km by 3 km) that was dominated by turtlegrass, with only minor presence (< 1% areal coverage) of other seagrass species or algae. Eliminating the shallow regions and patch reefs allowed us to reduce the habitat categories for further analyses to several categories of seagrass density and bare sand.

Replicate Sampling

We wanted to determine whether the change in seagrass coverage was consistent across the study area and provide a statistical basis for the study, so 40 ha replicates were used as subset samples. Twenty-four replicate sites, each about 4.0 ha (200 m by 200 m; 9.9 acres) in size, were chosen from the two images (48 sites total). The 24 replicates were at identical locations in each image, and were chosen at random (random-numbers generation by Excel). The choice of replicate size was made after checking the accuracy levels of classifying replicates between 1-25 ha, and finding a dip in

TABLE 1. *Data on seagrass characteristics and change in habitat. South Water Caye Marine Reserve, Belize.*

	Coverage by seagrass	Coverage by sand
2001 image		
Hectares	2352.3	430.5
Acres	5812.2	1063.6
%	84.5	15.5
2005 image		
Hectares	2300.0	480.1
Acres	5683.2	1186.4
%	82.7	17.3
Change in Habitat		
Hectares	-52.3	+49.6
Acres	-129.0	+122.8
%	-1.8	+1.8

accuracy level of replicate-sample sizes > 4 ha. The 4 ha replicates were placed on the image directly to the south of the randomly generated points. Classifying the 48 units (2 for each image) by numerous habitat categories during preliminary work allowed for a more accurate overall classification by heightening spectral differences in the areas of particular interest. Analysis of paired replicates from the two images allowed for precise quantification of changes, and provided detailed information on the geometry of habitat gain or loss that may not be obtained through the use of random (unmatched) replicates.

Results

The first objective was to determine areal expanse of seagrass, and address its change over time. For this we used a broad-scale analysis across the SWCMR, an area that supported 2,352.3 ha (5,812.2 acres) of seagrass during 2001 when the first image was taken (Table 1). Seagrass covered most (84.5%) of the seabed. Generally, some seagrass occurred across the entire study area, with the broadest areas of bare sand in the middle region, just north of the mangrove islands called Twin Cayes (Figure 2). Even those sandy regions had patches of seagrass scattered across them. North and south of the sandy region were extensive seagrass meadows, often represented as a mosaic of seagrass in varying densities.

Much of the study area had rounded patches of dense seagrass over a backdrop of more sparse seagrass habitat. The size of dense patches varied greatly, from < 10 m^2 to > 1 km^2 (near Tobacco Range; northern region). The backdrop of sparse seagrass also varied in spatial scale, usually grading from very sparse to moderate density seagrass habitat surrounding patches of bare sand. During 2001, the seagrass habitat of the region was measured at 53% dense seagrass, 18% moderate, and 29% sparse seagrass. Ground-truth data provided evidence that many regions were characterized by clear delineations between the various densities of seagrass, rather than the gradual-density increases that usually accompanied increasing depth.

Masking was required over 4% of the area to correct for cloud cover of the 2005 image. No masking was required for other features (e.g., patch reefs, back-reef areas), because those habitats were de-selected prior to classification. The

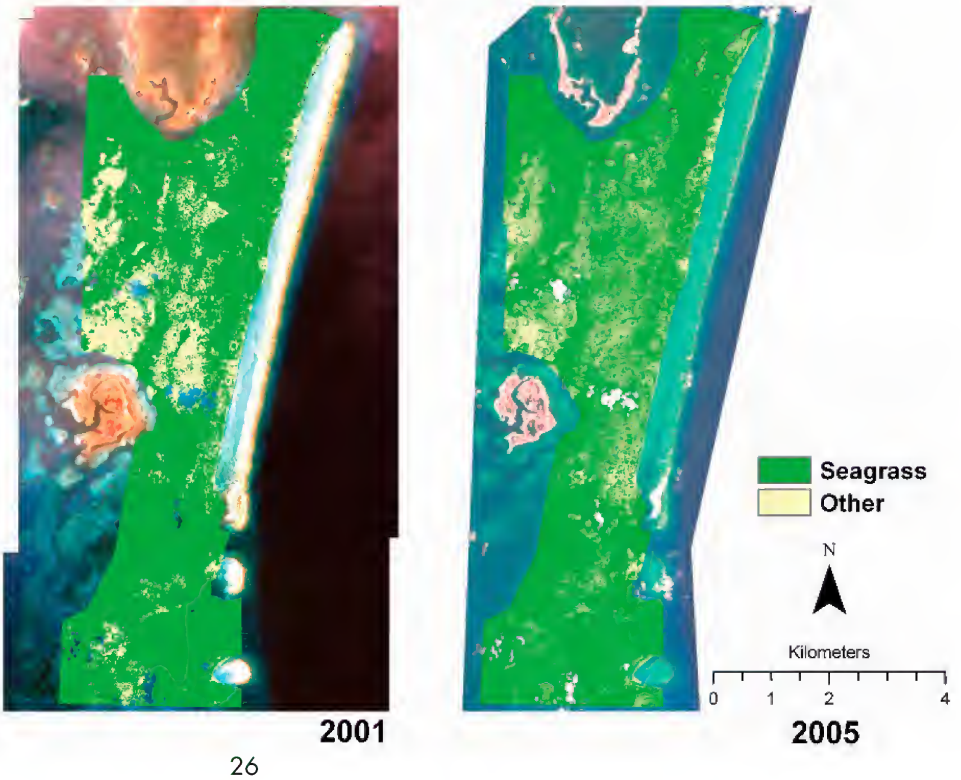

Figure 2. *Study area in the South Water Caye Marine Reserve, Belize. These two satellite images were taken during 2001 and 2005 to compare and contrast seagrass-habitat change. Seagrass (green) and sandy ocean floor (beige) are highlighted following classification techniques as areas of research focus. The land mass in the lower-middle of each image is Twin Cayes, a pair of mangrove islands. Tobacco Range, a circular loop of mangrove islands, occurs at the top of the image. The sandy areas of the 2005 image were muted by file decompression to jpeg (Joint Photographic Experts Group), so sand appears less distinctive than in the 2001 image.*

cloud mask rendered the images comparable in potential seagrass habitat, so that cloud-cover differences had no effect on our results.

The temporal component of the first objective required classification and quantification of seagrass in both images and comparisons of the results. All seagrass classified in the two images (all densities combined) was totaled to determine gain or loss overall. There was a loss of about 52.3 ha (129 acres) of seagrass from the study region, which represented a net decrease of 1.8% in seagrass habitat from 2001 to 2005 (Table 1). Dense seagrass decreased by 2.95%. Those data mean that overall there was a modest loss of seagrass across the region of study, from near Tobacco Caye to Curlew Caye. These analyses were corroborated by a corresponding increase in bare-sand seabed during 2005, and were verified through ground-truth data. A comparison of the two images, showing seagrass coverage, is provided in Figure 2.

The second objective, to determine whether seagrass gains or losses were consistent and significant across the study area, required sampling at randomly selected sites. We used 24 replicate samples (4 ha each) to assess potential medium-scale changes in seagrass coverage. The replicate samples confirmed a significant decrease in seagrass across the overall region of the SWCMR (1 way ANOVA; $p = 0.044$; $df = 46$).

Within the study area there were specific regions of significant seagrass loss. One 600 ha (1,483 acres) region in the middle of the study area, identified by replicates marked with stars in Figure 3, lost about 12.4% of its seagrass ($p = 0.017$; $df = 18$) during 4 years. A 240 ha region southeast of Twin Cayes, identified by dark circles (Figure 3), lost 40% of its seagrass ($p = 0.011$; $df = 6$). Although there were replicate samples with much seagrass gain, none of the regions that we analyzed with various spatial scales were statistically significant.

During preliminary work we analyzed the region by seagrass density. The accuracy assessments of those data were too low (89% for 2001; 90% for 2005) to warrant continued estimations of density as a measure of habitat loss, so we reduced the scope of detection to presence or absence of seagrass. This gave us a dataset with 500 observations of ground-truth data for each image, and only 3 errors in image classifications (all in the 2005 image) during an accuracy assessment. The reduced scope resulted in high accuracy-assessment values (> 99%).

The replicate samples provided high-resolution comparisons of various sites within the study area. Figure 4 is an example to indicate the flexibility and precision possible in assessing these remote-sensing data. There was 7.3% loss of seagrass at this location, as evidenced in red (middle image in Figure 4). We were able to analyze and quantify the entire study area in this manner.

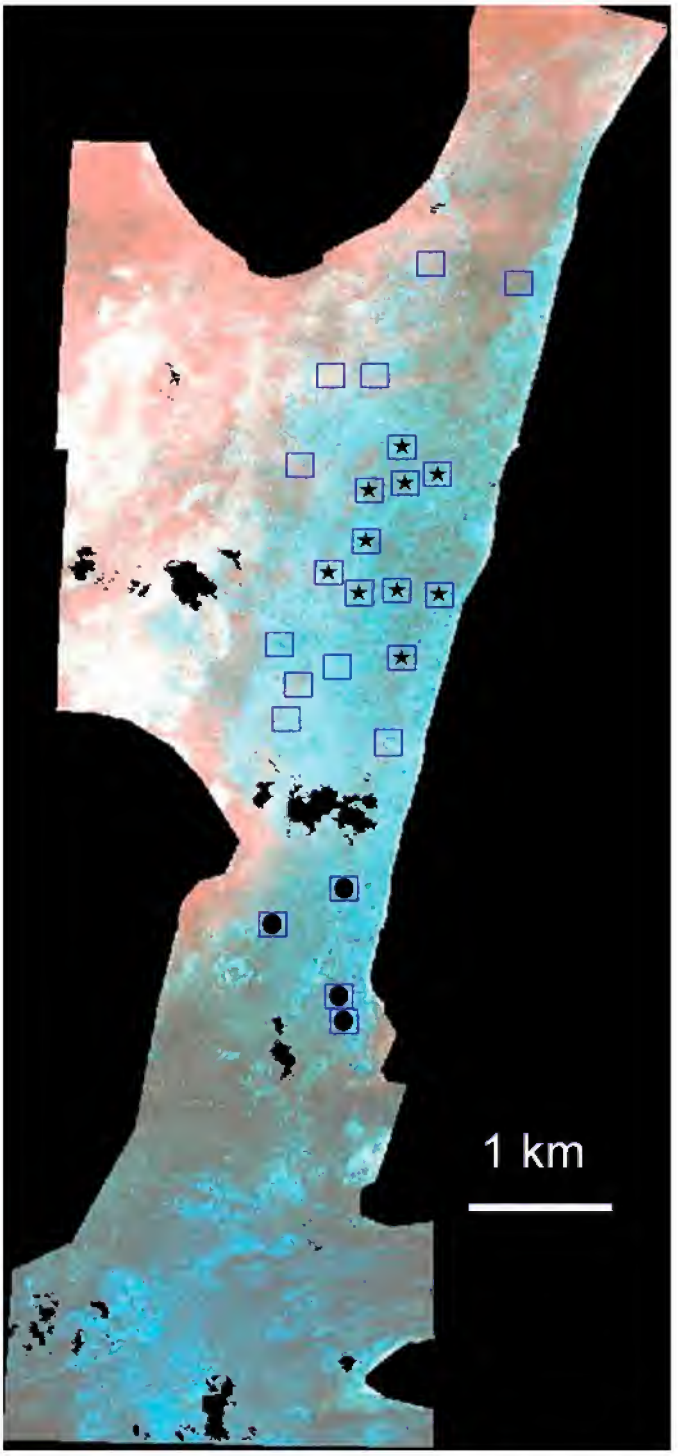

Figure 3. *Satellite image of the study area in the South Water Caye Marine Reserve, Belize. Replicates selected for statistical analyses are indicated by open squares. Replicates marked with black stars (12.4% seagrass loss) and circles (40% seagrass loss) are regions of significant change in seagrass. Clouds over the region appear as blackened areas with rough edges.*

Discussion

The decrease in seagrass across a broad-scale area of relatively pristine habitat in the South Water Caye Marine Reserve, and loss of 40% seagrass in some regions during a relatively short-duration study of 4 y, is reason for con-

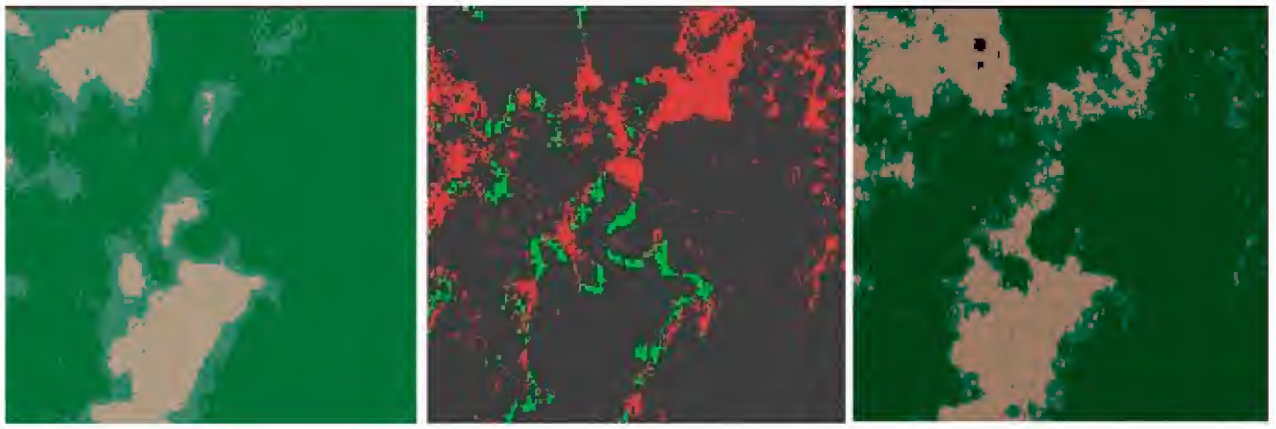

Figure 4. *One of the randomly selected areas showing an example of seagrass-habitat change. This image shows habitats classified into categories of dense seagrass (dark green), sparse seagrass (light green), and sand (beige) for 2001 (left) and 2005 (right). The middle image represents seagrass loss (red), gain (green), or no change (black) over the 4 y period. The coverage area shown is 200 m by 200 m.*

cern. These results, however, are not unique. Our results corroborate patterns of seagrass loss seen throughout many regions of the world (e.g., Short and Short 2003, Duarte et al. 2008).

SeagrassNet is a world-wide monitoring effort that includes some monitoring sites in Belize (Short et al. 2007). SeagrassNet sites were established along the coastal lagoon near Placencia (30 km SW of SWCMR) and Glover's Caye lagoon (35 km east of SWCMR). Short et al. (2006) reported a significant decrease in seagrass percent (46%) cover and shoot density (66%) near Placencia. They suggested that increased shoreline development was the likely cause. None of the study sites for SeagrassNet was placed in the SWCMR. Whereas other monitoring projects, such as SeagrassNet, used transect lines across the seagrass for data collections (Short et al. 2005), the satellite images we used provided data across an entire region. These remotely collected data had extensive flexibility for analysis, provided detailed information on several spatial scales, and allowed temporal comparisons of paired images in identical locations.

The use of remote sensing for seagrass studies is not new. Larkum and West (1990) used historical aerial photographs to find that 58% of the seagrass habitat in Botany Bay, NSW, Australia was lost over a 50 y period. Many other authors used remote sensing to monitor changes of seagrass (e.g., Ferguson and Korfmacher 1997, Hochberg et al. 2003, Duarte et al. 2008). The resolution of satellite imagery has increased greatly in recent years, which allows greater precision and accuracy than was available previously. Improved computers have allowed efficient handling of the huge datasets produced by these remote images. For instance, the original files for our 2005 image occupied 3 GB, which were too large for analysis by personal computers until recently.

The high accuracy assessments of our images can be attributed to the relatively high number of observations made in support of the classification data. This accuracy also benefitted from the simplicity of our habitat distinctions, once we merged the 60 classes into two contrasting habitats: seagrass versus sand. When we reduced the scope of detection to presence or absence of seagrass, and eliminated the shallow regions around the perimeter of the study area, we eliminated the errors almost entirely.

A change in seagrass habitat of 1.8% may not raise concern until one realizes that the value represents a habitat loss (seagrass to sand) across an area of 52.3 ha in 4 y, and likely an adverse effect on the organisms that inhabited that region. More salient to habitat concerns were the areas with rapid habitat loss identified in the replicate sampling, with significant regions of loss in excess of 12% and 40% (Figure 3). Seagrass that is replaced by sand means a 3 dimensional habitat was replaced by a more 2 dimensional habitat, with concurrent loss of refuges from predation, and degradation of the community (see Bolger et al. 2000).

Ground-truth observations of shrinking patch size confirmed the pattern of seagrass loss in the region, and was critical to interpreting the medium-scale patterns in seagrass change indicated by our replication study. Divers recorded that few of the seagrass patches in the region of 12% loss (Figure 3) had any rhizomes at all extending out into the sand from the established seagrass, indicating that expansion of patches was not occurring. These seagrass patches were very different in appearance from actively growing patches seen in northern portions of the SWCMR, where elongate rhizomes characterized the seabed, and long blades on the patch perimeter were typical. The short blade length that characterized patch perimeters of this area was further evidence that seagrass patches were shrinking in size. These blades appeared to be eroded by physical wearing or shortened by herbivore grazing.

The focus of this study was to determine whether seagrass habitat was being lost or gained, rather than address causes of change. But worldwide data and our preliminary analy-

ses provided insight into possible factors that affected the region. The most common factors related to seagrass loss worldwide are increased nutrient loading and greater turbidity (Cambridge et al. 1986, Carlson et al. 1994, Duarte 1995, Frankovich and Fourqurean 1997, Heck et al. 2000). Preliminary research in the study area indicated that the proximity of the broad seagrass meadows to deep channels (connections to clear, open-ocean water) was correlated (Wally and Gaston 2004). Those data demonstrated an inverse relationship between habitat patch size (perhaps fragmentation) and channel proximity, suggesting that water-quality measures (e.g., nutrients and turbidity) were related to the habitat loss in the SWCMR. The northern region of SWCMR has a wide, deep channel (12 m) near Tobacco Caye; the southern region near South Water Caye has a similar channel (10 m deep) (Figure 1). Both channels provide access for ocean-water flux into the lagoon during rising tides. Indeed, the large area of seagrass loss indicated by our replicate sampling (Figure 3), behind a 9 km barrier of reef, occurred almost midway between the two channels. This pattern leads us to suggest that the lack of clear, ocean water influx may be a limiting factor to survival of seagrass in that region.

The most salient result of our study was not the magnitude of seagrass loss *per se*, but the loss in seagrass habitat from an area that was thought to be relatively pristine. Unlike many broad-scale and long-term studies, we detected the loss of seagrass over a short period, by quantifying changes across the study area with high-resolution images. This resolution allowed detection of medium-scale changes, and the ground truthing provided confirmation and evidence of their accuracy. Our evidence of seagrass decline should stimulate action from those dependent on the ecosystem for their own wellbeing. The SWCMR was established during 2005, in part to protect its habitats from further decline. Our data were presented to the Belize Ministry of Fisheries and Coastal Management during a 2006 conference, and elicited concern from their personnel for the future of the region. The health and productivity of the coral-reef ecosystem in Belize depends on the quality and quantity of seagrass habitats in the region, as it does for similar ecosystems worldwide. Seagrass-habitat loss in this region of the Caribbean Sea may be evidence that even near-pristine areas can be impacted by anthropogenic factors. Finding out what led to decline of these habitats may help prevent loss of productivity by the ecosystem, and loss of ecological services to their associated human and nonhuman communities.

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Determining Salinity-Tolerance of Giant *Salvinia* Using Chlorophyll Fluorescence

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DETERMINING SALINITY-TOLERANCE OF GIANT SALVINIA USING CHLOROPHYLL FLUORESCENCE

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ABSTRACT: *Salvinia molesta* Mitchell, a floating invasive aquatic plant, is one of the top 10 worst invasive aquatic weeds in the world. It was discovered in the lower Pascagoula River in 2005 and evidence suggests that this non-native species is spreading along the northern Gulf of Mexico. These plants exhibit rapid growth and nutrient uptake rates, allowing them to out compete other plants in similar habitats. Distributional observations suggest that non-native *S. molesta* is able to survive in salinities of up to 7 ppt in the lower Pascagoula River. The response of *S. molesta* to three salinity levels (0, 5, 10 ppt) was tested using chlorophyll fluorescence. The health of the plants was measured over a period of one month, using a log scale series of observation intensities (hourly, daily, weekly). Plant responses indicated an acute salinity effect after about 4-6 hrs and then a gradual chronic decline. Compared to initial measurements, the final actual quantum yield (ΔF/Fm') dropped by 5%, 6% and 29%, while the final potential quantum yield (Fv/Fm) dropped 6%, 27% and 39% in the 0, 5, and 10 ppt treatments, respectively. Only plants in the 0 ppt treatment showed significant new growth. Plants in 5 ppt appeared to maintain themselves, but plants at 10 ppt all exhibited signs of severe stress and loss of color, turgor, and tissue viability after 10 d. Tolerance to brackish salinities has been reported in the past, and has implications for the use of the biological control agent, the weevil *Cyrtobagous salviniae*, that can only tolerate freshwater conditions.

INTRODUCTION

Aquatic plants can be grouped into three types: emergent, floating, and submerged (Pieterse and Murphy 1993), with some of the most successful invasive aquatic plants being in the floating group (e.g., *Eichhornia crassipes* and *Salvinia molesta*). These plants exhibit rapid growth rates, rapid nutrient uptake rates, are aggressive, and are competitive species that can impact aquatic environments, local economies, and human health (Holm et al. 1977). The impact of these species on a freshwater body is dramatically illustrated by *S. molesta*, one of the top 10 worst non-native invasive aquatic weeds in the world (Room and Julien 1995, Carley and Brown 2006). *Salvinia molesta* has become a world-wide problem, with invasions into freshwater bodies in most tropical countries, and was introduced into the United States in 1995 (Julien and Tipping 2002, USGS 2005).

Salvinia molesta has a doubling time of 4-6 d (Mitchell and Tur 1975) and was found in the lower Pascagoula River in 2005 (MS DMR 2005). Evidence reported in McFarland et al. (2004) suggests that this non-native species is spreading into the northern Gulf of Mexico (GOM). In Alabama and Mississippi, there are many suitable habitats for native and non-native invasive aquatic plants; there are four river drainage systems along the 121 km (75 mile) coastline of the state of Mississippi alone. The largest of these is the Pascagoula River, which holds the distinction of being the longest un-dammed, natural river remaining in the continental USA and provides habitat for numerous important and endangered salt marsh species (Schueler 2002). Much of this river system remains relatively unimpacted by development, except for the very lower reaches between the towns of Gautier and Pascagoula, MS.

Distributional observations during an outbreak in 2005 by personnel with the Mississippi Department of Marine Resources (DMR) suggest that non-native *S. molesta* was able to survive in salinities of up to 7 parts per thousand (ppt) in the lower Pascagoula River; a similar tolerance has been reported earlier by Divakaran et al. (1980) from growth tests conducted on salinities of 0 to 11 ppt. This has implications for the use of the biological control agent *Cyrtobagous salviniae* on this infestation, as this weevil can only tolerate freshwater conditions (Thomas and Room 1986, Julien et al. 2002). This observation is distressing in two respects: (1) potential for a portion of the non-native *S. molesta* population in the Pascagoula River to escape biological control; and (2) a more salinity-tolerant variety of this species could easily spread into similar habitats that abound along the GOM and elsewhere.

Pulse amplitude modulated (PAM) fluorescence is a tool to measure photophysiological processes *in vivo*. While it cannot be used to directly measure the mechanisms of osmoregulation, it has been used successfully to demonstrate the physiological stress resulting from salinity change in a number of aquatic plant species (Ralph 1998, Kamermans et al. 1999, Murphy et al. 2003, Biber 2006). PAM fluorescence has been used in submerged aquatic plants to measure acute stress, such as desiccation (Adams and Bates 1994, Bjork et al. 1999), temperature or salinity shifts (Ralph et al. 1998, Ralph 1999), and even changes in ambient light over short

time durations (Beer and Bjork 2000, Major and Dunton 2002). However, this technique has not been evaluated in floating aquatic plants subject to salinity stress, and at the time of the early S. *molesta* research in the 1970-80s this tool was not available. This study aims to determine both the efficacy of using a PAM fluorometer on this invasive aquatic plant, as well as to confirm earlier work of Divakaran et al. (1980) on the salinity tolerance of this species. Specifically, the aim of this study was to test the ability of giant salvinia (*S. molesta*) to tolerate salinities of 5 and 10 ppt using the PAM fluorescence technique.

Materials and Methods

The responses of non-native S. *molesta* plants (about 7 g dry weight) collected from the Pascagoula River delta (30°25.523' N, 88°34.640' W) were tested in three salinity levels (0, 5, 10 ppt). Plants recovered from field collecting and transport for 10 d prior to starting the experiment by being held in freshwater with adequate light; by this time plants were producing new leaves.

For each salinity level, a glass aquarium was placed under a 60W grow-light and a bank of fluorescent lamps (min 250 $\mu E\ m^{-2}\ s^{-1}$ PFFD) on a 12:12 hour cycle, in a growth chamber. Temperature was recorded in the 0 ppt and 10 ppt treatments every 15 min for the duration of the experiment by Hobo Tidbit recorders. Salinities were achieved by mixing de-ionized (DI) water with GF/F filtered (0.7µm) estuarine water of 20 ppt salinity. Water was charcoal filtered and circulated using aquarium filters (Whisper 10i) and allowed to condition for 10 d prior to plants being introduced into the three salinity treatments. Salinities were tested twice weekly using a refractometer and adjusted with DI water to replenish evaporative losses.

Chlorophyll fluorescence of photosystem II (PSII) was measured by the PAM technique. This provides an instantaneous measure of the effective quantum yield (ΔF/Fm') of PS II under prevailing ambient light conditions. In addition, photo-inhibition or quenching was also determined by measuring the potential quantum yield (Fv/Fm) of dark-adapted samples (Genty et al. 1989). Samples can be dark adapted with leaf-clips that are supplied with the PAM instrument. These are attached to a plant leaf and serve to occlude a small area of the leaf and then a shutter built into the clip is opened, exposing the leaf area under the clip to very low intensity red light transmitted through fiber-optics. The chlorophyll in the dark-adapted area of the leaf fluoresces and the initial fluorescence (Fo) is recorded. Upon illumination with a high intensity burst of saturating light through the fiber-optics, the pigments associated with PSII become overwhelmed and the maximal fluorescence (Fm) is recorded. The difference between the maximal and initial fluorescence levels (Fm - Fo) is called the variable fluorescence (Fv) and from this the ratio Fv/Fm, or potential quantum yield is calculated. In an analogous fashion the effective quantum yield ΔF/Fm' can be determined on leaf samples that are not dark adapted and are exposed to ambient light.

On the day prior to the salinity stress experiment (day 0), 30 leaves were haphazardly sampled, one per plant, for actual quantum yield (ΔF/Fm') and 6 leaves were sampled for potential quantum yield (Fv/Fm) after a minimum of 5 min dark adaptation time using the leaf clips and fiber optic supplied with the mini-PAM (Walz, Germany). Default instrument settings were used and measurement intensity (gain) was set to 1; these settings were maintained for the duration of the experiment. The population was then separated into three equal portions, determined by blotted wet weight (g).

On the morning of day 1, plants were sequentially introduced into the three experimental salinity treatments and one leaf per plant was selected for analysis. Immediately on immersion into the salinity treatment, 6 leaves were dark-adapted using the clips for Fv/Fm, then ΔF/Fm' measurements were taken on 30 leaves that were not dark adapted. When the measurements on the 30 leaves were complete, the 6 clipped leaves had dark-adapted and were also measured. All 36 measurements typically took less than 15 min to complete.

Every hour after the initial introduction, the cycle of 30 ΔF/Fm' and 6 Fv/Fm measurements were repeated for each of the three salinities on haphazardly selected plants. Hourly PAM measurements were continued for 12 hrs (8 am to 8 pm). The following day, and every day for the next 7 d, PAM measurements were repeated between 11-12 am. After the first week, any plants marked for new leaf growth were checked to count new leaf production. Over the next three weeks, plant fluorescence was measured at least once per week at the same time of day, and weekly observations on new-leaf production were recorded.

Chlorophyll fluorescence variation due to leaf age was tested in the control (0 ppt) mid-way through the experiment (day 16). PAM measurements were made in duplicate on each leaf pair on 15 individuals. Additionally, leaf size, shape, and color for each leaf pair was noted. Leaf age was denoted as immature (small green leaves formed apically), mature (large green leaves distal to the new leaves) and senescent (large leaves with discoloration and loss of turgor). From these data, mean fluorescent yield values by leaf age were determined to better understand within-plant variation.

At the end of the experiment, all plant material was removed from each tank and sorted into newly formed green leaves and original leaves. Five representative leaves were saved from each of the three salinity treatments for chlorophyll analysis using standard methods (Arar 1997). Wet weight (g) of the remaining biomass was recorded after blotting the sample dry. Samples were dried at 60-70 °C and reweighed to determine dry weight (g).

Statistical analysis was done in JMP (SAS Institute, Cary,

N.C.) using either a T-test or ANOVA as appropriate. Prior to the test, data were determined to satisfy the assumptions of normality and homoscedasticity; data transformation was not necessary. Significant results ($p < 0.05$) were followed by a Tukey's honestly significant difference posthoc test, and significantly different means are grouped by superscripts on the figures.

Results

Some variation occurred in the treatment salinities because of evaporation in the growth chamber. The 0 ppt treatment remained in the optimal range of <1ppt throughout the one month duration of the experiment. The salinity in the 5 ppt treatment ranged from 5-7 ppt and there was 30% less growth compared to the 0 ppt treatment. The salinity of the 10 ppt treatment ranged from 9-12 ppt, representing the upper lethal tolerance.

During day 1, the light adapted yield (ΔF/Fm') exhibited a slight, but nonsignificant, decline during the first 4 h in the 0 ppt and 5 ppt treatments, with mean fluorescence dropping from 0.736 at the first reading, down to 0.690 and 0.705, respectively, before recovering again (Figure 1a). There was a significant (T-test: $t_{1,8} = 1.86$, $p = 0.005$) drop in ΔF/Fm' in the 10 ppt treatment after 4 h, from 0.716 to 0.518, with an increase back to fluorescence values similar to other salinities for the remainder of day 1 (Figure 1a). In contrast, the dark-adapted fluorescence yield, Fv/Fm, ranged between 0.770 and 0.820 for all three salinity treatments with very little change over the 12 h (Figure 1b).

During the remaining four weeks of the experiment, both ΔF/Fm' and Fv/Fm measurements showed similar responses by salinity treatment, although ΔF/Fm' was more variable. After day 5, there was a decline in chlorophyll fluorescence across all treatments with Fv/Fm dropping slightly from 0.813 to 0.778 by the end of week 1, and ΔF/Fm' declining from a mean of 0.738 to 0.712 (0 ppt), 0.732 (5 ppt), and 0.680 (10 ppt). Chlorophyll fluorescence was lower during week 2 than week 1, with ΔF/Fm' showing greater day-to-day fluctuations than Fv/Fm. At the end of the second week, ΔF/Fm' in the 10 ppt treatment was lower (0.663) than the other two treatments (Figure 1c). The greatest difference among salinity treatments became evident in the last two weeks of the experiment. In the 10 ppt treatment, both ΔF/Fm' and Fv/Fm declined substantially to ending values of 0.517 (Figure 1c) and 0.498 (Figure 1d), respectively. In

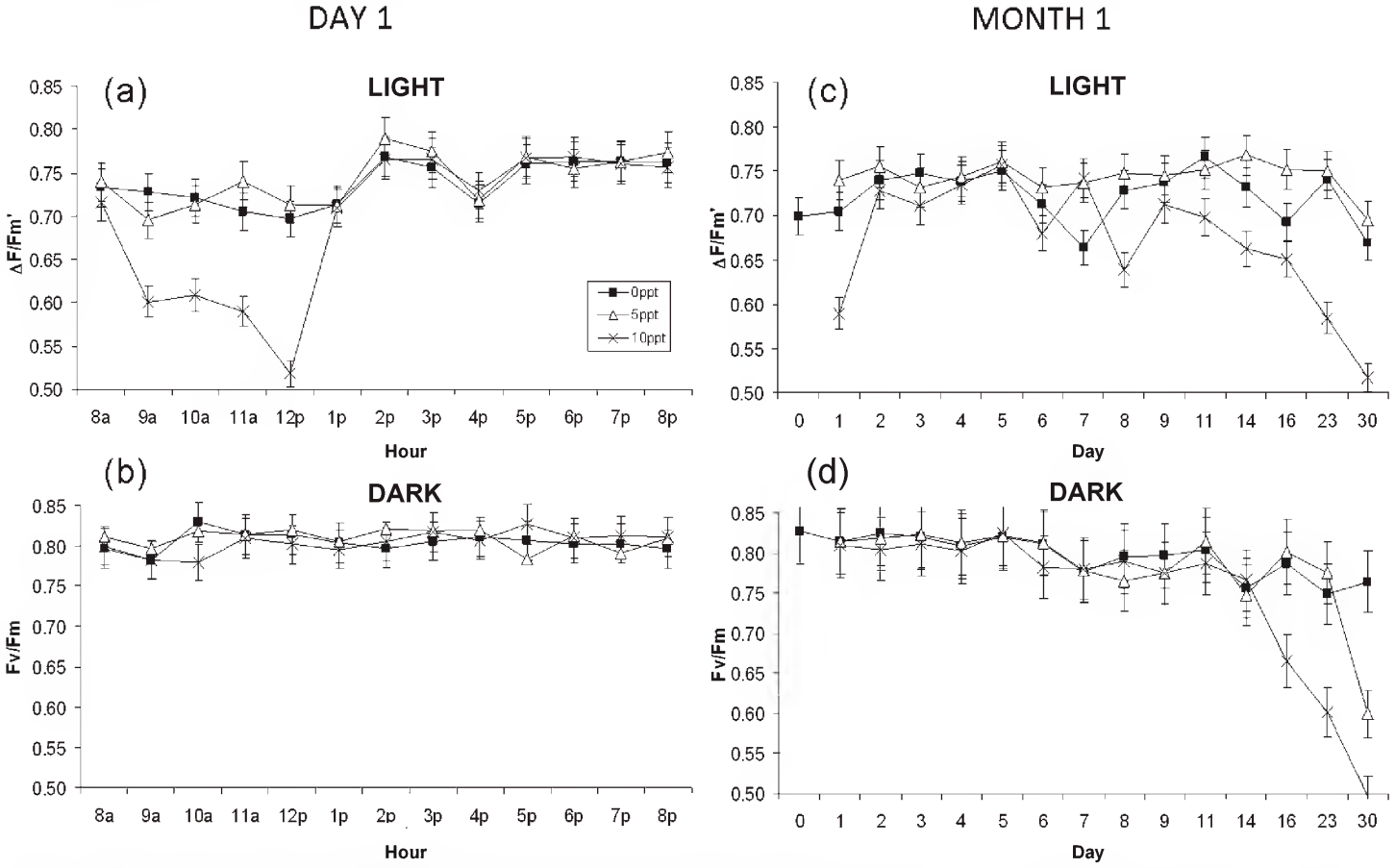

Figure 1. *Mean (± 1 se) chlorophyll fluorescence over time in* S. molesta *plants exposed to three different salinities (0, 5, and 10 ppt).*
(a) Hourly effective quantum yield (ΔF/Fm') on 30 leaves on day 1
(b) Hourly potential quantum yield (Fv/Fm) from 6 dark-adapted leaves on day 1
(c) Noon-time effective quantum yield (ΔF/Fm') on 30 leaves from day 1 to day 30
(d) Noon-time potential quantum yield (Fv/Fm) from 6 dark-adapted leaves from day 1 to day 30.

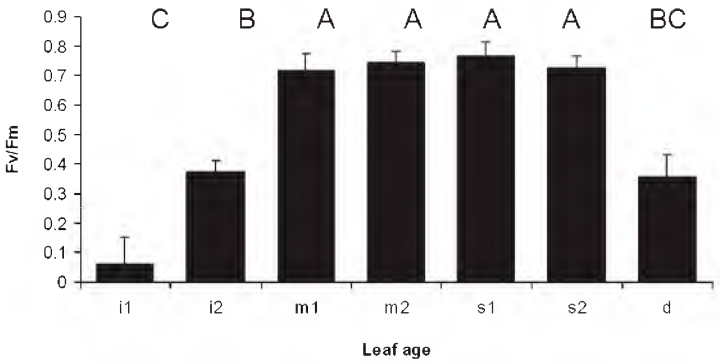

Figure 2. *Mean (± 1 se) dark-adapted yields (Fv/Fm) measured on day 16, for leaves of different ages in 0 ppt. For each age, leaves were grouped into a smaller and a larger size class (1 = 0.3-1.0 cm, 2 = 1-3 cm mid-rib length). i1 and i2—immature green leaves, m1 and m2—large mature green leaves, s1 and s2—senescing brown leaves, d—black dead leaves. Superscript letters indicate significantly different means from Tukey's HSD posthoc test.*

the 5 ppt plants, Fv/Fm also dropped to 0.599 in the last week of the experiment (Figure 1d). Compared to initial measurements, the final light-adapted yields were 95%, 94% and 71%, while the final dark-adapted yields were 94%, 73% and 61% in the 0, 5, and 10 ppt treatments, respectively.

PAM fluorescence was found to vary significantly by leaf age (one-way ANOVA: $F_{6,47} = 20.21$, $p < 0.001$). Immature and dead leaves had significantly lower Fv/Fm values compared to mature green and senescing leaves; Fv/Fm was ≥ 0.700 in mature and senescing leaves, and < 0.500 in all others (Figure 2). Immature leaves were smaller (<1 cm) in size than mature leaves (>1 cm mid-rib length).

Initial biomass in the three treatments was less than 1.63% different, with 1.73 g total dry weight in the 0 ppt, 1.71 g in 5 ppt and 1.74 g in the 10 ppt treatments, respectively (Figure 3a). Final biomass decreased with increasing salinity, and a 40.64% decline was noted in the 10 ppt compared to the 0 ppt treatment. There was 1.78 g total dry weight in the 0 ppt, 1.22 g in 5 ppt and 1.06 g in the 10 ppt treatments, respectively. New leaf growth contributed 0.235, 0.031, and 0 g dry weight in the 3 salinity treatments (Figure 3a). The plants exhibited some withering and decrease in leaf production at 5 ppt, and a gradual but incomplete senescence with no new leaf production at 10 ppt.

Leaf chlorophyll concentration was significantly different among treatments (one-way ANOVA: $F_{3,20} = 49.11$, $p < 0.001$). Plants in the 0 ppt treatment had significantly higher chlorophyll concentrations with 9.69 ± 3.97 µg/ mg in new leaves and 2.83 ± 1.31 µg/mg in old leaves, compared to only 1.51 ± 0.22 µg/mg in senescent leaves in the 10 ppt treatment (Figure 3b). The chlorophyll concentrations corresponded with observations of leaf color and tissue integrity among the three treatments.

Discussion

Observations made on the non-native *S. molesta* plants over the course of the experiment indicated that higher salinity caused loss of turgor, pigments, and a reduction in new leaf formation. At the end of the first week, the plants in the 5 ppt treatment showed signs of browning, especially in the older leaves, and had fewer new leaves. In the 10 ppt treatment, the leaves had folded together, most of the green color was lost, and no new leaves were produced. At the end of the experiment, the 0 ppt treatment had substantial new leaf growth with 1-2 new leaf pairs per individual; all leaves were at least partly green in color. The 5 ppt treatment had minimal new leaf growth and the leaves were a darker green with some brown. In the 10 ppt treatment there was no new leaf growth and the remaining leaves were brown to black and had lost turgor.

Health of *S. molesta* has been determined in previous studies by assessing the general appearance of the individuals, pigment content and changes in texture of the leaves, the rate of decay and disintegration, as well as the production of new growth (Divakaren et al. 1980, Finlayson 1984). Since those studies, the technique of chlorophyll fluorescence has become widely adopted as a robust and reliable technique, easy to carry out, non-destructive and rapid (Maxwell and Johnson 2000, Ralph et al. 2007). In general, the greater

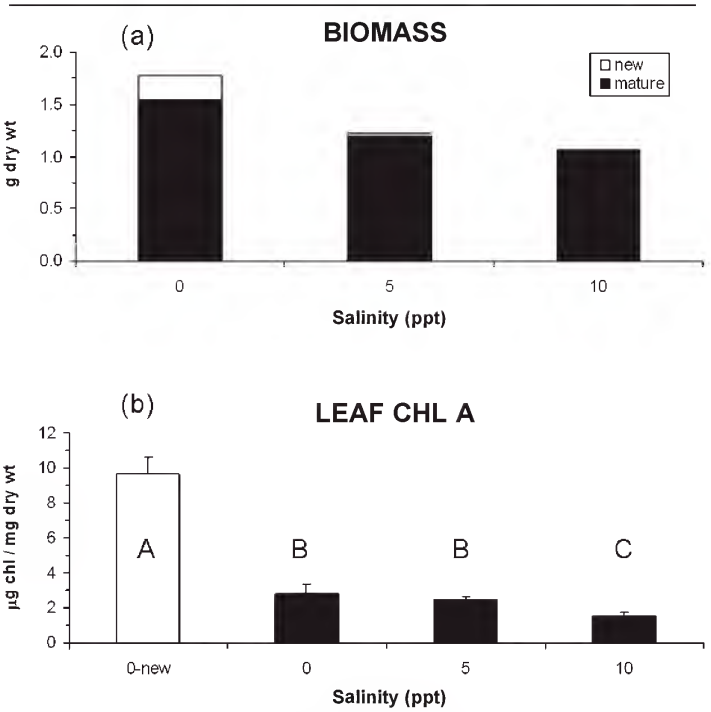

Figure 3. *Biomass and chlorophyll content of new (immature) and mature leaves in 3 salinity treatments.*
(a) Final dry weight of all plant material. Biomass was separated into new immature leaves produced during the experiment, and mature leaves that were present at the beginning.
(b) Mean (± 1 se) leaf chlorophyll content of immature leaves (0 ppt only) and mature leaves. Superscript letters indicate significantly different means from Tukey's HSD posthoc test.

the quantum yield of chlorophyll fluorescence as measured using PAM Fv/Fm, the higher the efficiency of the light reactions in photosynthesis, which equates to a plant under low physiological stress. Generally, the maximum possible proportion of the solar energy absorbed into photosynthesis is around 83% (Maxwell and Johnson 2000). Any decline in this ratio (either ΔF/Fm' or Fv/Fm) indicates a reduction in the efficiency with which light is converted to photosynthetic product and subsequently, growth or reproductive output, and such a decline is often seen when a plant becomes stressed (Krause and Weis 1991, Rohacek and Bartak 1999).

The stressor that was tested in this study was hyperosmotic stress in the non-native aquatic invasive *S. molesta*, which grows optimally in freshwater (Mitchell et al. 1980, Room and Gill 1985). In a previous study (Divakaran et al. 1980), the growth of this species was reduced by 25% at 3.5 ppt and growth was "very slow" at 7 ppt. Salinities above 7 ppt were reported to be unfavorable, with total withering taking place at salinities of 11 ppt and above (Divakaren et al. 1980). At lethal salinities of 11 ppt and above, *S. molesta* becomes more and more spongy and soft in texture. The stem and "roots" shrink and the color of the leaves turns from green to brown (also noted in Divakaren et al. 1980). In this experiment, new leaves grew only in the 0 and 5 ppt treatments, suggesting that one of the responses to increased salinity stress by *S. molesta* is a reduced ability to produce new leaf segments. The implications of this are a reduction in ramet production causing a reduction in the potential number of clonal fragments, leading to reduced population growth over time.

The chlorophyll fluorescence data indicates that there was a decrease in "plant health" in all 3 salinity treatments over the course of the 1 mo trial, but that the decline was more pronounced at 10 ppt than in the other 2 treatments. Similar sub-lethal responses to salinity have been measured using chlorophyll fluorescence in submerged aquatic (Ralph 1998) and emergent wetland plants (Biber 2006). *Salvinia molesta* may be able to tolerate salinities of 5 ppt and even produce new leaves, but salinities of 10 ppt greatly stress the plants and they are unable to survive after prolonged exposure of more than 3 weeks. This coincides with previous data based on physical appearance and growth rate. These results suggest that *S. molesta* is not able to tolerate elevated salinities > 10 ppt for prolonged periods of time (> 1 mo). However, at salinities around 5 ppt, typical of the lower Pascagoula River where this invasive non-native aquatic was found, these plants did demonstrate the ability to maintain photosynthesis and new leaf growth for at least one month. Additional studies on the long term persistence of this species at salinities present in the lower Pascagoula are warranted to better understand the ability of *S. molesta* to persist in the absence of specific management actions.

These findings are cause for concern, as they indicate that *S. molesta* may be able to persist in the low salinity conditions typical of the upper reaches in many northern GOM estuaries. Further, it could be possible for this invasive non-native aquatic to establish populations in higher salinity conditions than the non-native biological control agent, *Cyrtobagous salviniae*, a freshwater weevil (Thomas and Room 1986, Julien et al. 2002), exacerbating difficulties in controlling *S. molesta* outbreaks. Other control options include herbicide applications, which can also affect native plants, or manual control, which is generally not cost-effective (Pieterse and Murphy 1993). For this reason, the Mississippi DMR commenced a comprehensive eradication program using herbicides, which appears to have been successful at controlling *S. molesta* in this outbreak (Dale Diaz, pers. comm., Mississippi Department of Marine Resources, Biloxi).

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Morphological Characteristics of Early Life History Stages of the Blue Crab, *Callinectes sapidus* Rathbun, from the Northern Gulf of Mexico with a Comparison of Studies from the Atlantic Seaboard

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MORPHOLOGICAL CHARACTERISTICS OF EARLY LIFE HISTORY STAGES OF THE BLUE CRAB, *CALLINECTES SAPIDUS* RATHBUN, FROM THE NORTHERN GULF OF MEXICO WITH A COMPARISON OF STUDIES FROM THE ATLANTIC SEABOARD

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ABSTRACT: Zoeae, megalopae, and early crab stages of *Callinectes sapidus* Rathbun, 1896 were described from the northern Gulf of Mexico (nGOM). Observations during this study were based on larvae reared in the laboratory through the early crab stages and on megalopae and early crab stages collected in the wild. Gulf of Mexico data are compared with similar information for the southeast Atlantic coast of the United States. Size and setation of *C. sapidus* larvae reared from nGOM stocks were different than those in published descriptions of larvae reared from Atlantic populations. Seasonal differences in size were noted in both reared and wild caught specimens. Zoeal stages I, II and III of larvae cultured in the spring were larger than corresponding larvae hatched in the summer/fall. Data sets on zoeal stages IV and V were incomplete; however, seasonal differences in measurements on all structures tended to be smaller in the summer/fall reared larvae. No seasonal differences were observed for the sixth and seventh zoeal stages, megalopal stage and first crabs. Spring reared larvae had higher survival rates when reared at optimal temperature (25°C) and required fewer zoeal stages (6) to reach the megalopal stage. Megalopae and first crabs collected from the plankton exhibited distinct seasonal variations and were larger in the spring than in fall.

INTRODUCTION

The blue crab, *Callinectes sapidus*, supports large commercial and recreational fisheries along the Atlantic coast and in the northern Gulf of Mexico (nGOM) (Guillory et al. 2001). The ability to reliably separate blue crab larvae from other portunids is beneficial over a broad range of ecological and fishery-related studies of coastal plankton. However, identification of C. *sapidus* larvae from plankton samples in the nGOM is complicated by the overlapping spawning periods among the large number of portunid species found in the region and the abundance and distribution of its sympatric congener, *Callinectes similis*. In addition, successful mass culture of blue crabs in Maryland and in Mississippi has generated interest in their culture for stock replenishment in the Chesapeake Bay and for expansion of the soft crab fishery in the nGOM (Zmora et al. 2005, Perry et al. 2005). Current aquaculture techniques require identification of specific larval stages to track development since feed size and type varies with zoeal stage.

Stuck and Perry (1982) provided characters useful in the identification of C. *sapidus* larvae and early crab stages, but these descriptions are not readily available in the general literature. The current study draws upon that early work and provides descriptions of developing blue crabs from the first zoeal stage through the second crab stage. Additionally, seasonal differences in morphology among larvae reared from nGOM populations are presented and the morphological differences in C. *sapidus* larvae reared from the nGOM are compared to existing data from the Atlantic coast. Data from this study will provide a means by which to track zoeal development in plankton collections and aquaculture operations in the nGOM.

MATERIALS AND METHODS

Egg bearing females were collected from the Mississippi Sound in cold water temperature (April 1980; spring) and again during warm water temperature (late August 1980; summer/fall) and brought into the laboratory for spawning and collection of newly hatched larvae. Larvae hatched from 6 different females (3 spring, 3 summer/fall) were isolated and reared separately in mass and individual cultures. All larvae were reared in 30 ppt filtered artificial seawater with Penicillin (60 mg/l) and Streptomycin (50 mg/l) added. Larvae from each seasonal group were reared at both optimal (25.0°C, Sandoz and Rogers 1944, Costlow and Bookhout 1959, Sulkin and Epifanio 1975) and ambient temperatures. Ambient spring temperatures, initially 16.0°C, were slowly increased at a rate of about 1.0°C/week to correspond to naturally occurring conditions. Ambient summer/fall temperatures were initially 30.0°C and were gradually decreased at a rate of 1.0°C/week. Photoperiod was held constant at 12L:12D. Depending upon the experimental temperature, the culture period ranged from about 3 -13 weeks.

Larvae from each hatch were initially isolated in a series of three mass culture bowls, each with about 500 larvae in

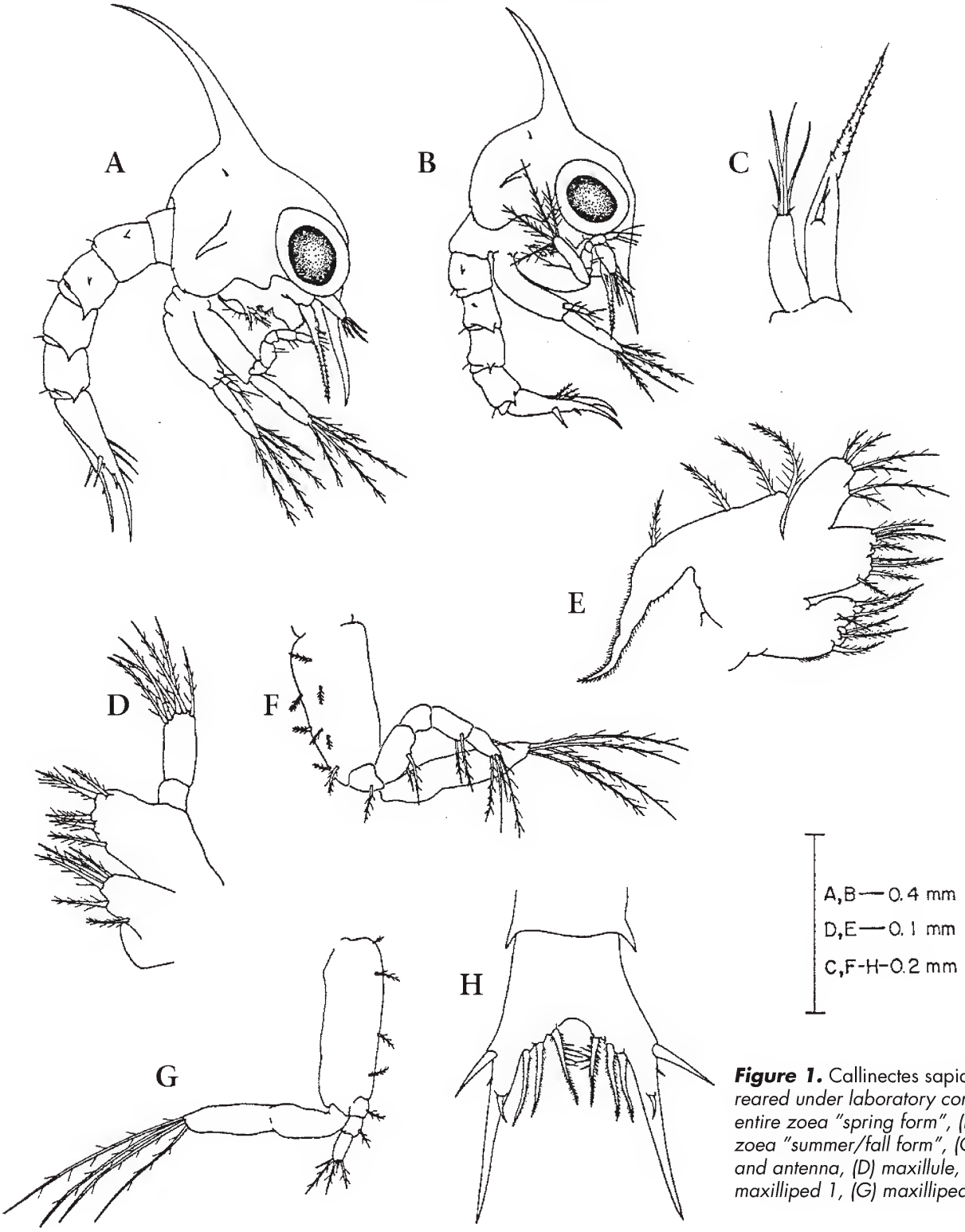

Figure 1. Callinectes sapidus *zoea I reared under laboratory conditions. (A) entire zoea "spring form", (B) entire zoea "summer/fall form", (C) antennule and antenna, (D) maxillule, (E) maxilla, (F) maxilliped 1, (G) maxilliped 2, (H) telson.*

1 L of seawater. Additional larvae (18) were placed in plastic boxes, one zoea per cubicle in 200 ml of seawater. Two identical sets of larvae were prepared from each parent, one reared at optimal and the other at ambient temperature for individual and mass culture. Zoeae were transferred to clean water daily and initially fed a diet of rotifers (first 14 d) and a combination of rotifers and brine shrimp after 2 weeks. Exuviae were removed and preserved in 5% formalin from both mass and individual cultures. Additional live zoeae from each developmental stage (about 20) were preserved in 10% formalin from mass cultures. Time required for larvae to become megalopae in mass cultures was determined by recovery of final stage zoeae exuviae from culture bowls. Percent survival to the megalopal stage was determined by daily observation of larvae in individual culture for each season and experimental temperature.

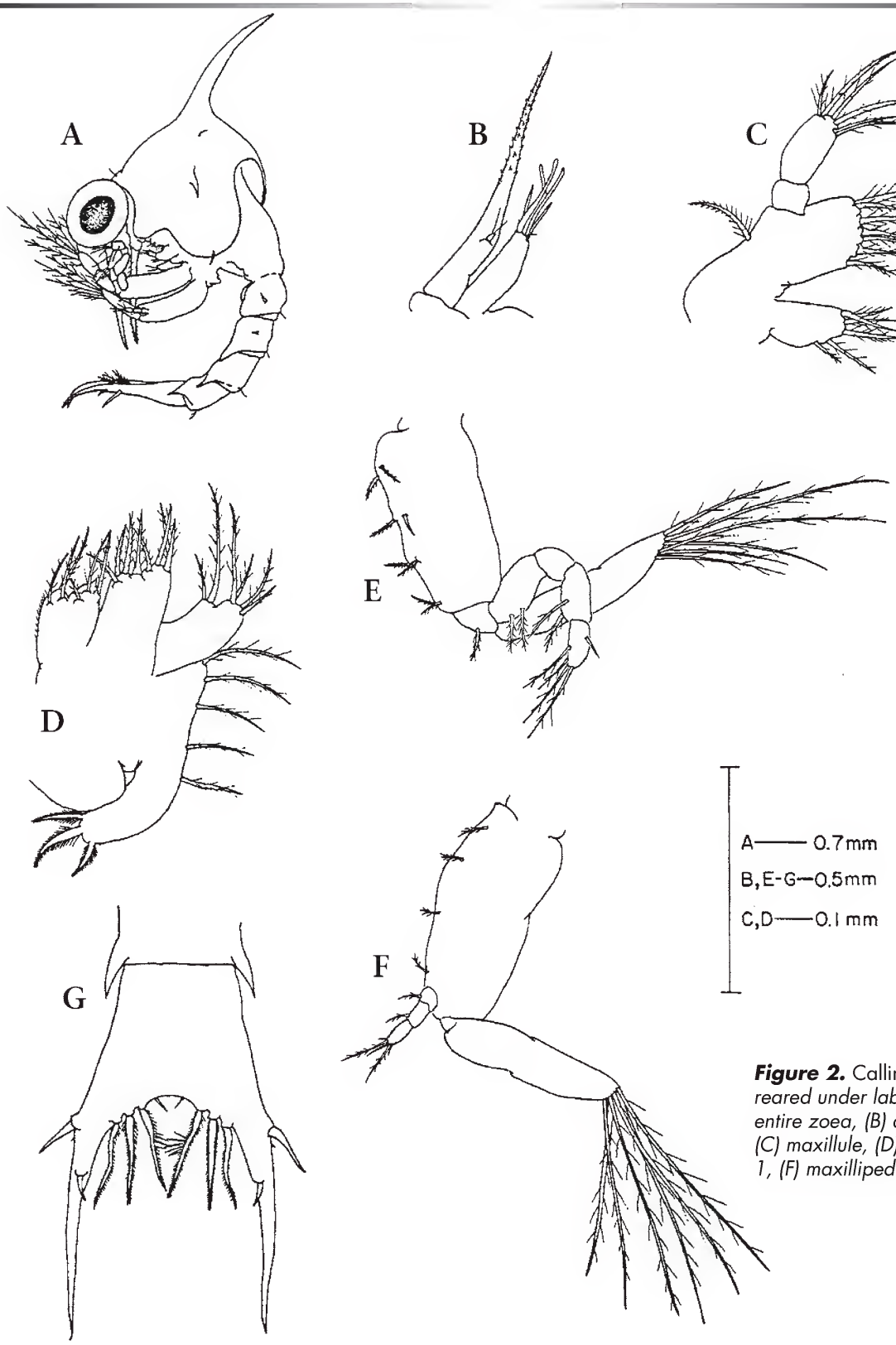

Figure 2. Callinectes sapidus *zoea II reared under laboratory conditions. (A) entire zoea, (B) antennule and antenna, (C) maxillule, (D) maxilla, (E) maxilliped 1, (F) maxilliped 2, (G) telson.*

Measurements (mm) were made on exuviae for a number of morphological characters of larvae obtained from each culture set for both spring and summer/fall broods. Measurements included total carapace length and width, dorsal and rostral spine length, and appendage setation for 10 larvae of each stage. Data were compiled as the mean and range for each character for each brood; raw data (individual measurements) were lost during Hurricane Katrina. All illustrations were made with a camera lucida.

Blue crab megalopae were collected in zooplankton samples taken from Dog Keys Pass, MS in May (spring) and October (fall) of 1980. These megalopae were cultured individually in the laboratory until they reached a size at which positive identification as *C. sapidus* could be made (crab stages 3-5). Measurements (mm) of total carapace length, rostral length, antennal length and carapace width for megalopae

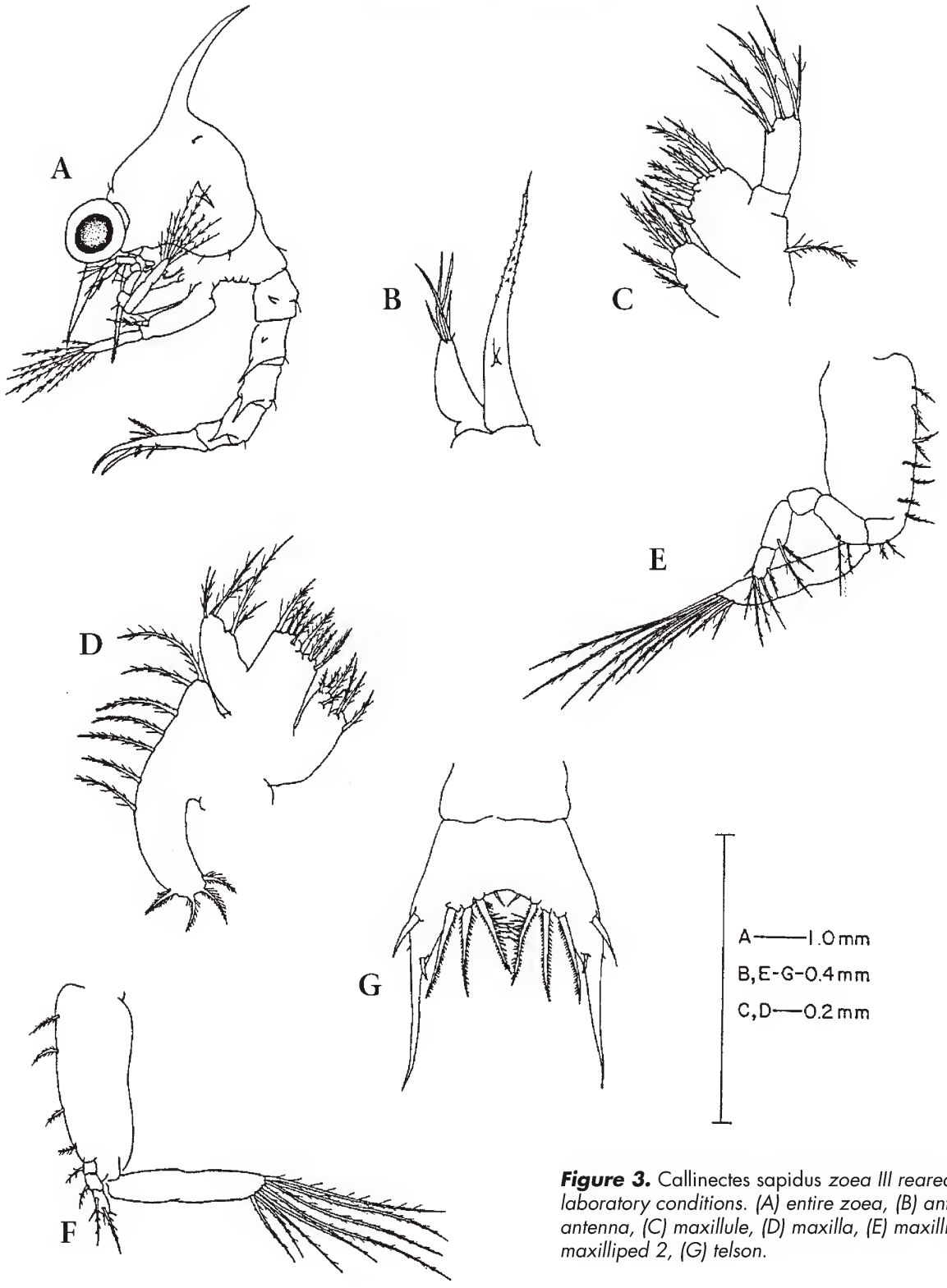

Figure 3. Callinectes sapidus *zoea III reared under laboratory conditions. (A) entire zoea, (B) antennule and antenna, (C) maxillule, (D) maxilla, (E) maxilliped 1, (F) maxilliped 2, (G) telson.*

and subsequent first crabs were made from the recovered exuviae.

Results

Survival, Rate, and Duration of Larval Development

The percent survival of individually held larvae reared through the megalopal stage varied with brood, temperature and season (Table 1). Larvae reared at 25.0°C in the spring showed the highest overall survival (44.5%), while larvae reared at 25.0°C in the summer/fall had the lowest survival (18.5%). Although quantitative survival estimates for larvae held in mass cultures were not made, trends were similar to those reported for larvae held in individual cultures.

Among larvae reared in individual culture, the number of zoeal stages required to reach the megalopal stage varied

TABLE 1. *Survival (%) of blue crab larvae reared from ovigerous females collected in spring and summer/fall and held in individual cultures. Survival is expressed as percent of larvae reaching the megalopal stage at each experimental temperature.*

	SPRING SPAWN		SUMMER/FALL SPAWN	
Temperature (°C)	**25.0**	**≥ 16.0**	**25.0**	**≤ 30.0**
Brood 1	38.9	27.8	22.2	44.4
Brood 2	38.9	16.7	27.8	61.1
Brood 3	55.6	66.7	5.5	11.1
Mean	44.5	37.1	18.5	38.9

from 6 to 9 (Table 2). Within the group of larvae reared in the spring, the majority reached the megalopal stage in 6 molts. In contrast, few of the larvae reared from summer/fall broods reached the megalopal stage in only 6 molts. Within a season there were no notable differences in the number of zoeal molts to the megalopal stage between optimal and ambient temperatures.

Time required for development of spring larvae cultured at ambient temperature (≥ 16.0°C) was about twice that of larvae reared at 25.0°C (Table 3). Spring larval groups held individually required a mean of 34.6 d to reach the megalopal stage when cultured at 25.0°C compared to 65.7 d when cultured at ambient temperatures. Additionally, the range of time for development was completely separate for the two temperatures (Table 3). Summer/fall larvae held individually at 25.0°C and ambient temperatures (≤ 30.0°C) required a mean of 44.3 and 46.6 d, respectively, to reach the megalopal stage, and the range in development times overlapped almost completely between temperatures. A minimum of 23 d was required to reach the megalopal stage for spring larvae (brood 3) held in mass culture at 25.0°C. Surviving spring larvae held in mass culture at ambient temperatures required a minimum of 52 d to become megalopae (brood 3). Minimum time required to reach the megalopal stage for summer/fall larvae held in mass cultures at 25.0°C and ambient temperatures was 37 and 32 d, respectively (brood 1).

Morphological Development and Description of Larvae

Seven zoeal and one megalopal stage were identified in the present study. While some larval series consisted of 6, 8 or 9 zoeal stages, the resulting "combined" or "additional" stages usually possessed a composite of morphological characters from the 7 types presented here. The pattern of the chromatophores was identical for all zoeal stages and similar to that described by Costlow and Bookhout (1959).

Zoea I. Total length 0.90-1.25 mm (Figure 1)

Carapace (Figure 1A, B): smooth with prominent dorsal, lateral and rostral spines, minute seta present on each side at base of dorsal spine, posterior ventral margin with 1 or 2 fine serrations. Rostral spine 0.20-0.30 mm, dorsal spine 0.30-0.48 mm, carapace spine width 0.45-0.67 mm (measured at base), carapace spine length 0.79-1.17 mm. Eyes unstalked.

Antennule and antenna (Figure 1C): antennule conical, bearing 3 aesthetascs, 1 or 2 slender setae and 1 or 2 minute "hair-like" setae (seen only under extreme magnification). Protopodite of antenna slender, bearing 2 rows of minute spines on distal half; exopodite small, terminating with 2 simple setae of unequal length.

Maxillule (Figure 1D): endopodite with 2 articles, distal article with 4 terminal and 2 slightly subterminal plumose setae; basal endite of protopodite bearing 5 plumose setae; coxal endite with 5 or 6 plumose setae.

Maxilla (Figure 1E): scaphognathite with 4 marginal setae on distal portion, proximal portion tapered; endopodite unarticulated, bearing 4 terminal and 2 subterminal setae; basal endite of protopodite with 7 or 8 plumose setae; coxal endite with 3 (rarely 2) plumose setae on each lobe.

Maxilliped 1 (Figure 1F): exopodite with 4 terminal plumose setae; basal article of endopodite with 8 (rarely 7 or 9) plumose setae, distal 5 articles usually with plumose setae arrangement of 1, 2, 0, 2, 5.

Maxilliped 2 (Figure 1G): exopodite with 4 terminal plumose setae; basal article of endopodite with 4 setae; distal 3 articles with plumose setae arrangement of 1, 1, 4 or 5 (2 long, 2 or 3 short).

Abdomen and telson (Figure 1A-B, H): abdomen consisting of 5 segments and a telson; second segment with a short knob-like process on each dorsal lateral surface, third seg-

TABLE 2. *Number of zoeal stages (indicated by Roman numerals) required to reach the megalopal stage listed by season, experimental culture temperature and brood. Numbers in parentheses indicate the actual number of complete larval series recovered from individual cultures and included in the analysis.*

	SPRING SPAWN		SUMMER/FALL SPAWN	
Temperature (°C)	**25.0**	**≥ 16.0**	**25.0**	**≤ 30.0**
Brood 1	VI (1) VII (6)	VI (2) VII (2) VIII (1)	VI (1) VII (4)	VI (1) VII (4) VIII (2) IX (1)
Brood 2	VI (3) VII (4)	VI (3)	VI (2) VII (2)	VI (1) VII (7) VIII (2)
Brood 3	VI (7) VII (3)	VI (8) VII (3)	VII (1)	VI (1) VII (1)
Overall	VI (24) VII (18) VIII (1)		VI (6) VII (19) VIII (4) IX (1)	

TABLE 3. *Time (in days) required for development of larvae through the megalopal stage in both individual (IC) and mass (MC) culture.*

SPRING SPAWN

Temperature (°C)		Brood 1 25.0	Brood 1 ≥ 16.0	Brood 2 25.0	Brood 2 ≥ 16.0	Brood 3 25.0	Brood 3 ≥ 16.0	Combined Data 25.0	Combined Data ≥ 16.0
Mean									
	IC	37.6	66.4	34.3	66.0	31.9	64.6	34.6	65.7
	MC	36.0	65.2	35.1	63.7	28.5	60.5	33.2	63.1
Range									
	IC	33-49	62-72	26-41	62-72	26-42	56-75	26-49	56-75
	MC	33-41	57-88	30-46	55-82	23-36	52-71	23-46	52-88

SUMMER/FALL SPAWN

Temperature (°C)		Brood 1 25.0	Brood 1 ≤ 30.0	Brood 2 25.0	Brood 2 ≤ 30.0	Brood 3 25.0	Brood 3 ≤ 30.0	Combined Data 25.0	Combined Data ≤ 30.0
Mean									
	IC	38.0	39.0	49.0	43.3	46.0†	57.5	44.3	46.6
	MC	48.0	40.3	46.1	47.3	49.5	48.0	47.9	45.2
Range									
	IC	33-46	32-48	40-59	32-52	46.0†	56-60	33-59	32-60
	MC	37-60*	32-50	40-60*	34-58	46-60*	37-56	37-60*	32-58

†Based on a single surviving larva.
*Terminated in final zoeal stage.

ment with a short hook on each side, segments 2 to 5 with pair of minute dorsal setae, segments 3 to 5 with prominent lateral spines; telson bifurcate ending in two acute spines, each furca with a single small dorsal seta and 1 large robust and 1 minute lateral seta, inner margin of each furca with 3 large, heavily armored robust setae.

Zoea II. Total length 0.94-1.45 mm (Figure 2)

Carapace (Figure 2A): similar to first zoea, but larger, bearing a single seta on anterior margin just above eyes and a single seta on posterior ventral margin, no serrations. Rostral spine 0.22-0.42 mm, dorsal spine 0.30-0.55 mm, carapace spine width 0.47-0.73 mm, carapace spine length 0.80-1.30 mm. Eyes stalked.

Antennule and antenna (Figure 2B): antennule with 3 aesthetascs and 1 to 2 setae. Antenna similar to first zoea.

Maxillule (Figure 2C): endopodite as in first zoea; most specimens with a large seta on inner margin of protopodite, basal endite with 7 plumose setae, coxal endite with 4 apical and 2 subterminal plumose setae.

Maxilla (Figure 2D): scaphognathite with 5 (rarely 4 or 6) distal marginal setae and 3 apical setae; endopodite similar to first zoea; basal endite of protopodite with 8 or 9 plumose setae, coxal endite with 3 plumose setae on each lobe, distal margin of inner lobe produced into a sharp seta.

Maxilliped 1 (Figure 2E): exopodite with 6 terminal plumose setae; endopodite similar to first zoea.

Maxilliped 2 (Figure 2F): exopodite with 6 terminal plumose setae; endopodite similar to first zoea.

Abdomen and telson (Figure 2A, G): abdomen similar to first zoea except lateral spines more developed. Telson similar to first zoea except for addition of a pair of slender setae to inner medial margin of furca.

Zoea III. Total length 1.12-1.80 mm (Figure 3)

Carapace (Figure 3A): similar to second zoea, but slightly larger, single seta added on rostrum between eye stalks, posterior ventral margin bearing 1 or 2 setae. Rostral spine 0.32-0.53 mm, dorsal spine 0.32-0.67 mm, carapace spine width 0.58-0.87 mm, carapace spine length 1.02-1.60 mm.

Antennule and antenna (Figure 3B): unchanged from second zoea.

Maxillule (Figure 3C): endopodite as in second zoea; all specimens with a large seta on inner margin of protopodite, basal endite with 8 or 9 plumose setae, coxal endite with 4 or 5 apical and two sub-terminal plumose setae.

Maxilla (Figure 3D): scaphognathite with 6 or 7 marginal plumose setae on distal portion, proximal margin with 4 (rarely 3) plumose setae; endopodite and protopodite similar to second zoea.

Maxilliped 1 (Figure 3E): exopodite with 8 terminal plumose setae; endopodite similar to second zoea except for the addition of a second subterminal seta on terminal article.

Maxilliped 2 (Figure 3F): exopodite with 8 terminal plumose setae; endopodite unchanged from second zoea.

Abdomen and telson (Figure 3A, G): abdomen consisting of 6 segments; pair of dorsal setae added to first 4 segments, sixth segment without lateral spine. Telson setation unchanged from second zoea.

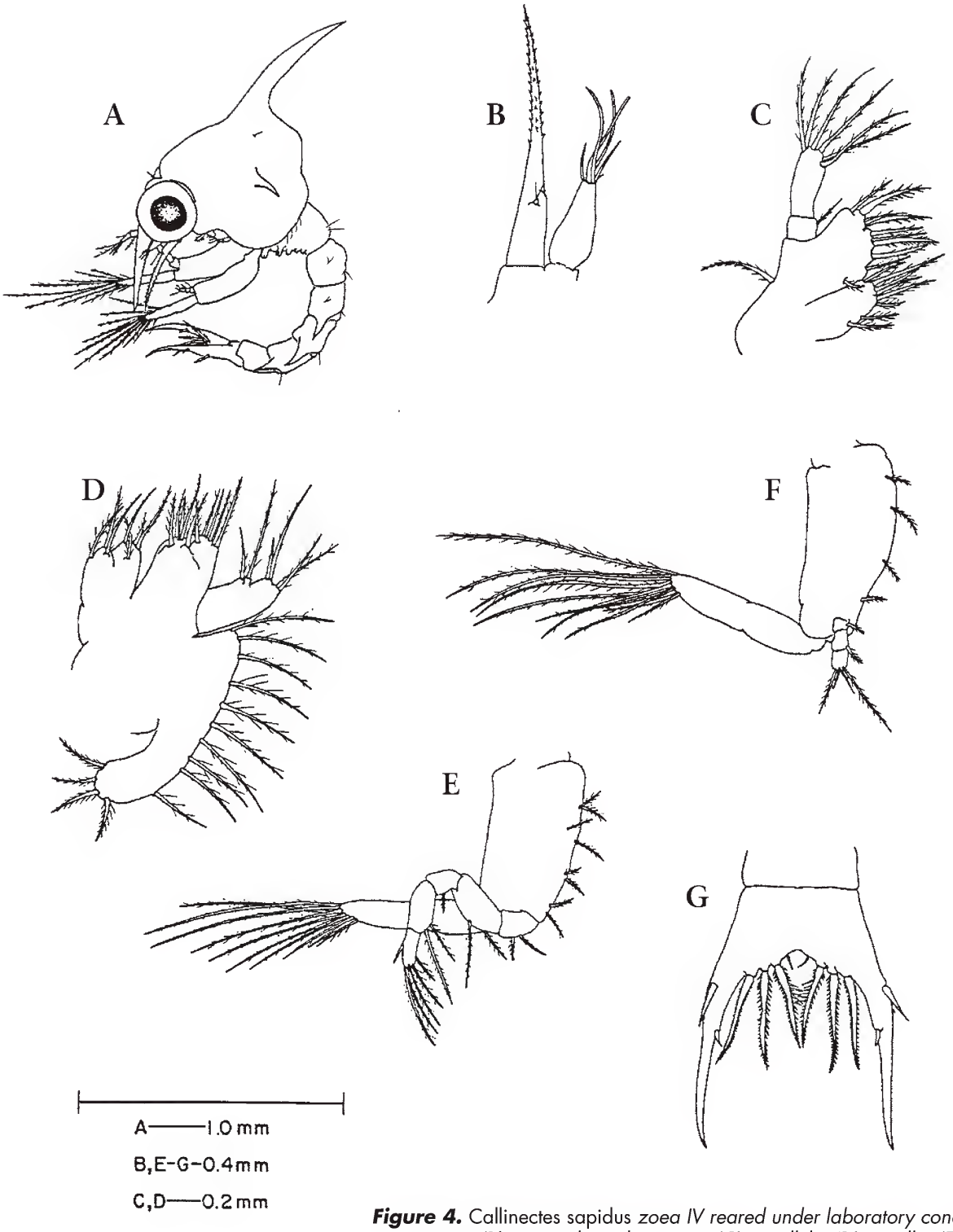

Figure 4. Callinectes sapidus *zoea IV reared under laboratory conditions. (A) entire zoea, (B) antennule and antenna, (C) maxillule, (D) maxilla, (E) maxilliped 1, (F) maxilliped 2, (G) telson.*

Zoea IV. Total length 1.50-2.15 mm (Figure 4)

Carapace (Figure 4A): similar to third zoea, but larger posterior ventral margin bearing 4 or 5 setae; buds of third maxilliped, chelae and pereiopods barely visible beneath carapace. Rostral spine 0.35-0.67 mm, dorsal spine 0.43-0.84 mm, carapace spine width 0.67-1.10 mm, carapace spine length 1.19-2.04 mm.

Antennule and antenna (Figure 4B): antennule unchanged from third zoea. Antenna with slight swelling just distal to insertion of exopodite marking development of endopodite bud.

Maxillule (Figure 4C): proximal article of endopodite armed with a single plumose seta, distal article as in third zoea; basal endite of protopodite with 9 terminal plumose se-

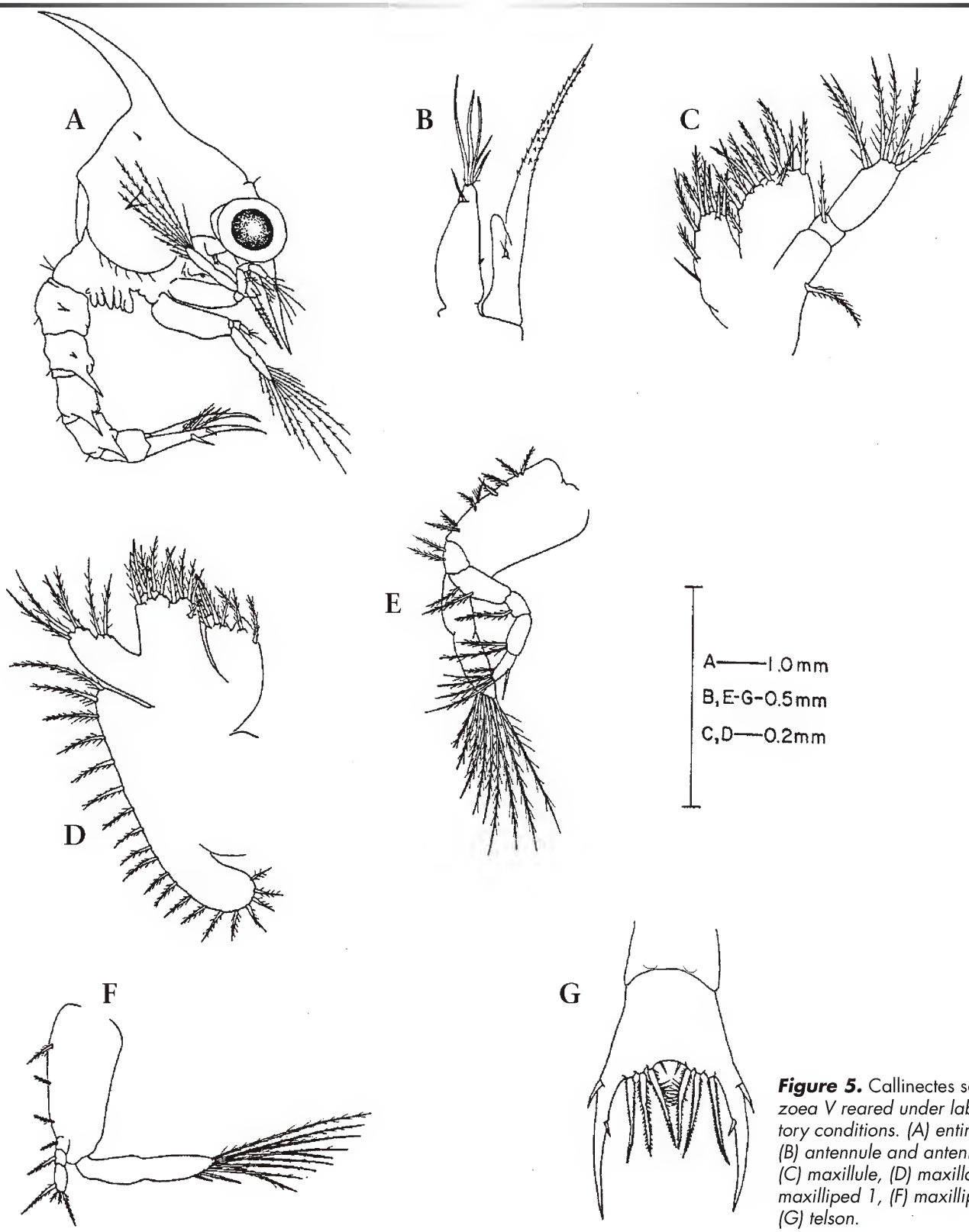

Figure 5. Callinectes sapidus *zoea V reared under laboratory conditions. (A) entire zoea, (B) antennule and antenna, (C) maxillule, (D) maxilla, (E) maxilliped 1, (F) maxilliped 2, (G) telson.*

tae and occasionally a single subterminal plumose seta, coxal endite with 5 or 6 terminal and 2 subterminal plumose setae.

Maxilla (Figure 4D): scaphognathite with 12 to 22 plumose setae along the entire length; endopodite unchanged from third zoea; basal endite with 9 to 10 plumose setae; inner lobe of coxal endite bearing 4 plumose setae, outer lobe with 3.

Maxilliped 1 (Figure 4E): exopodite with 9 or 10 (rarely 8) terminal plumose setae; endopodite with plumose setae arrangement of 2, 2, 1, 2, 6.

Maxilliped 2 (Figure 4F): exopodite with 10 (rarely 9) terminal plumose setae; endopodite unchanged from third zoea.

Abdomen and telson (Figure 4A, G): similar to third zoea.

Zoea V. Total length 1.70-2.40 mm (Figure 5)

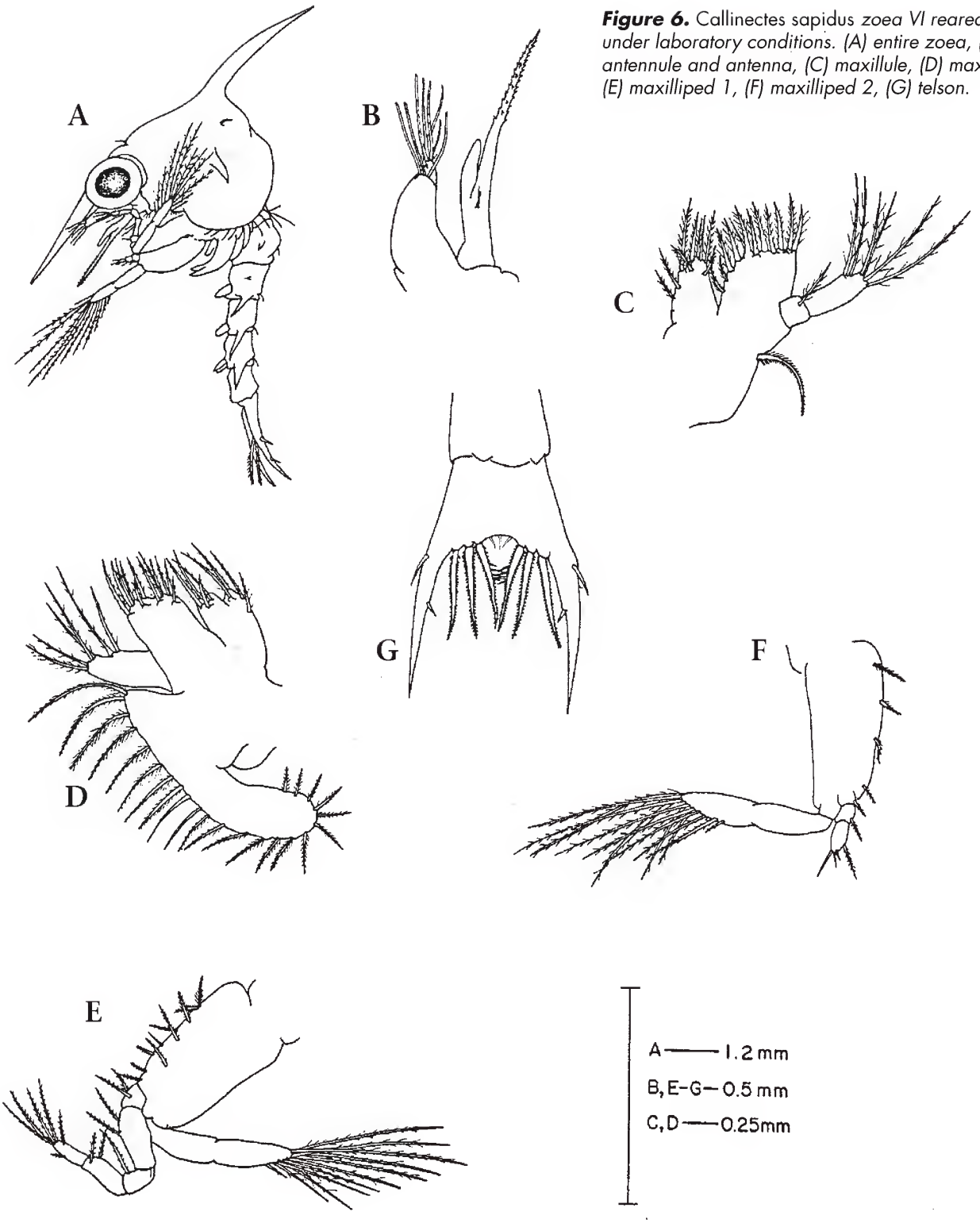

Figure 6. Callinectes sapidus *zoea VI reared under laboratory conditions. (A) entire zoea, (B) antennule and antenna, (C) maxillule, (D) maxilla, (E) maxilliped 1, (F) maxilliped 2, (G) telson.*

Carapace (Figure 5A): similar to fourth zoea but larger, posterior ventral margin bearing 6 (rarely 7) setae; buds of third maxilliped, chelae and pereiopods showing further development from fourth zoea. Rostral spine 0.42-0.78 mm, dorsal spine 0.58-0.97 mm, carapace spine width 0.85-1.17 mm, carapace spine length 1.60-2.30 mm.

Antennule and antenna (Figure 5B): antennule with 1 or 2 setae and 3 or 4 terminal aesthetascs plus 1 or 2 subterminal aesthetascs; antennal endopodite bud clearly visible, extending about one-sixth distance to tip of antenna.

Maxillule (Figure 5C): endopodite similar to fourth zoea; basal endite of protopodite with 11 or 12 total spines, coxal endite with 6 terminal and 2 or 3 subterminal spines.

Maxilla (Figure 5D): scaphognathite with 17 to 26 marginal

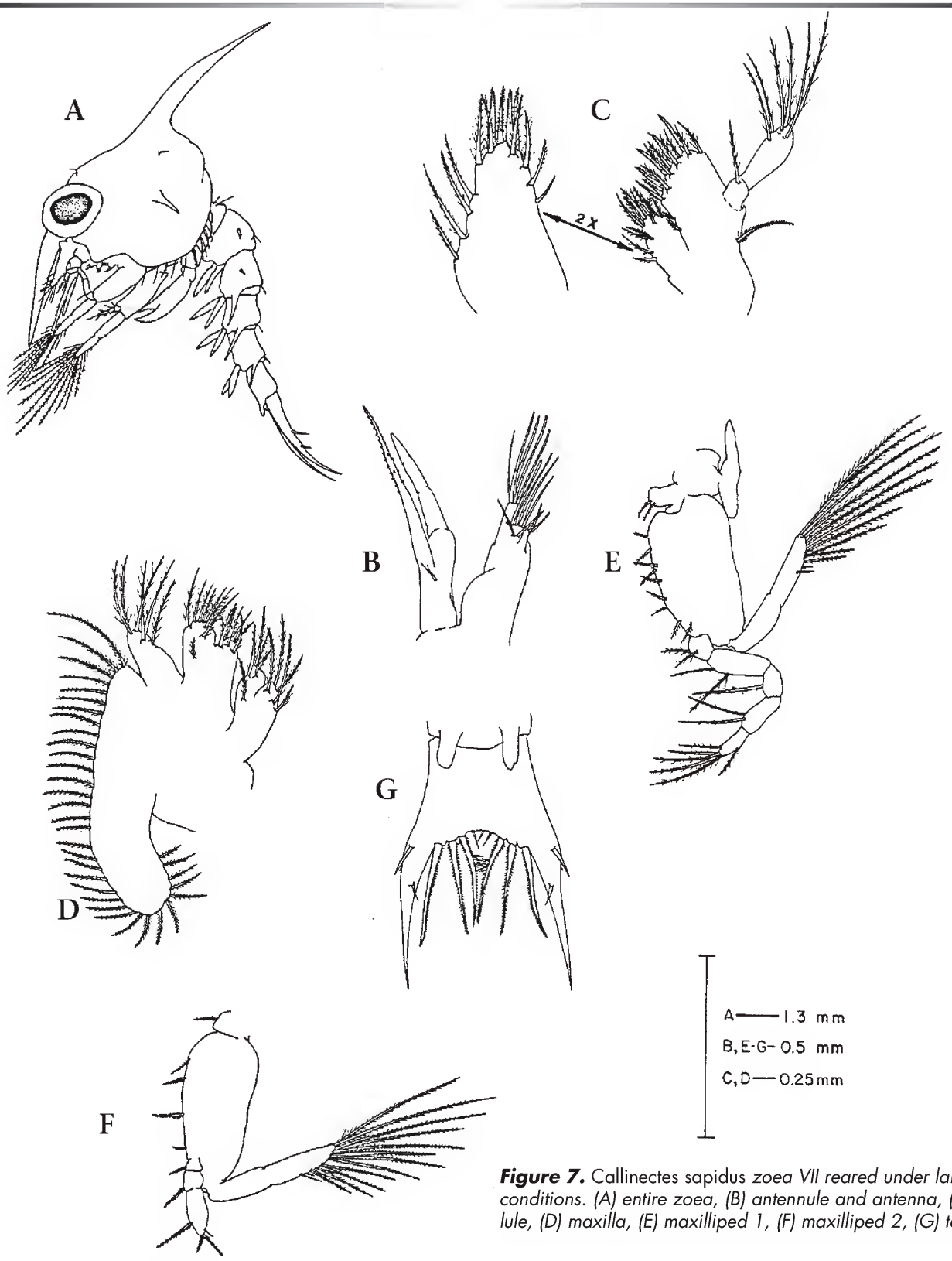

Figure 7. Callinectes sapidus *zoea VII reared under laboratory conditions. (A) entire zoea, (B) antennule and antenna, (C) maxillule, (D) maxilla, (E) maxilliped 1, (F) maxilliped 2, (G) telson.*

plumose setae; endopodite unchanged from fourth zoea; basal endite of protopodite with 12 total setae, coxal endite unchanged from fourth zoea.

Maxilliped 1 (Figure 5E): exopodite with 10 (rarely 9 or 11) terminal plumose setae; endopodite unchanged from fourth zoea.

Maxilliped 2 (Figure 5F): exopodite with 11 (occasionally 12) terminal plumose setae; endopodite unchanged from fourth zoea.

Abdomen and telson (Figure 5A, G): slight swellings on distal ventral margin of segments 2 to 5 indicating development of pleopods (visible on some specimens); 2 or 3 small setae present in telson furca; remainder of abdomen and telson similar to fourth zoea.

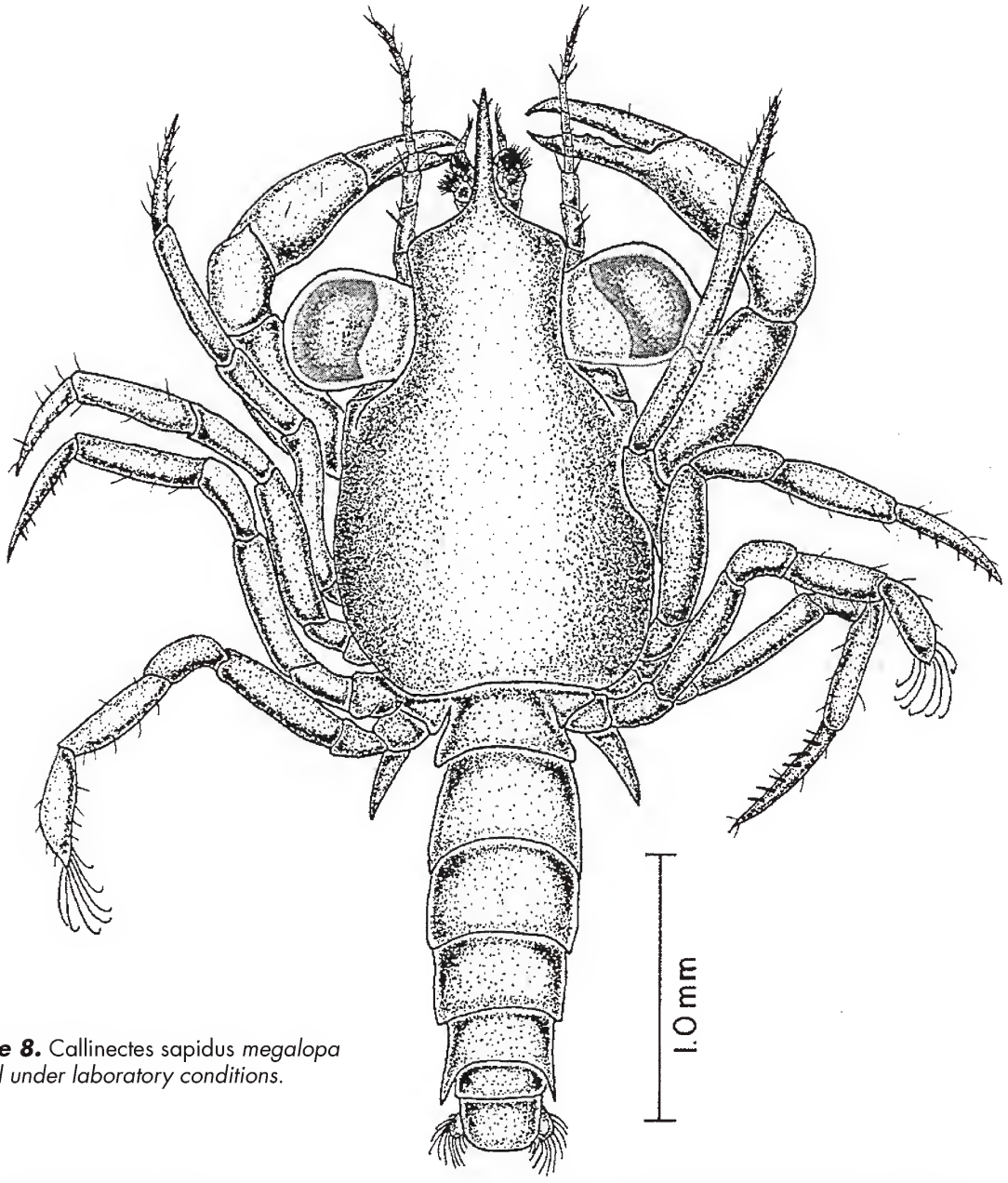

Figure 8. Callinectes sapidus *megalopa reared under laboratory conditions.*

Zoea VI. Total length 2.24-2.84 mm (Figure 6)

Carapace (Figure 6A): similar to fifth zoea but larger, posterior ventral margin bearing 9 setae; chela bud greatly lengthened with bifid distal tip, remaining appendage buds showing additional development from fifth zoea. Rostral spine 0.67-0.92 mm, dorsal spine 0.75-1.14 mm, carapace spine width 1.04-1.34 mm, carapace spine length 1.98-2.60 mm.

Antennule and antenna (Figure 6B): antennule with 1 or 2 setae and 3 or 4 terminal aesthetascs, second subterminal row of 2 or 3 aesthetascs added. Endopodite bud extending one-fourth to one-half distance to tip of antenna.

Maxillule (Figure 6C): endopodite similar to fifth zoea; basal endite of protopodite with 12 to 14 total plumose setae, coxal endite with 6 or 7 terminal and 2 or 3 subterminal plumose setae.

Maxilla (Figure 6D): scaphognathite with 22 to 30 marginal plumose setae; endopodite and basal endite of protopodite unchanged from fifth zoea; inner lobe of coxal endite with four plumose setae, outer lobe with 3 or 4 plumose setae.

Maxilliped 1 (Figure 6E): exopodite with 11 (rarely 10 or 12) terminal plumose setae; basal article of endopodite with 9 or 10 setae; endopodite unchanged from fifth zoea.

Maxilliped 2 (Figure 6F): exopodite with 12 (rarely 11 or 13) terminal plumose setae; endopodite unchanged from fifth zoea.

Abdomen and telson (Figure 6A, G): pleopods clearly visible on abdominal segments 2 to 5, barely visible on segment 6. Inner medial margin of telson furca with 3 (rarely 2) small setae, otherwise unchanged from fifth zoea.

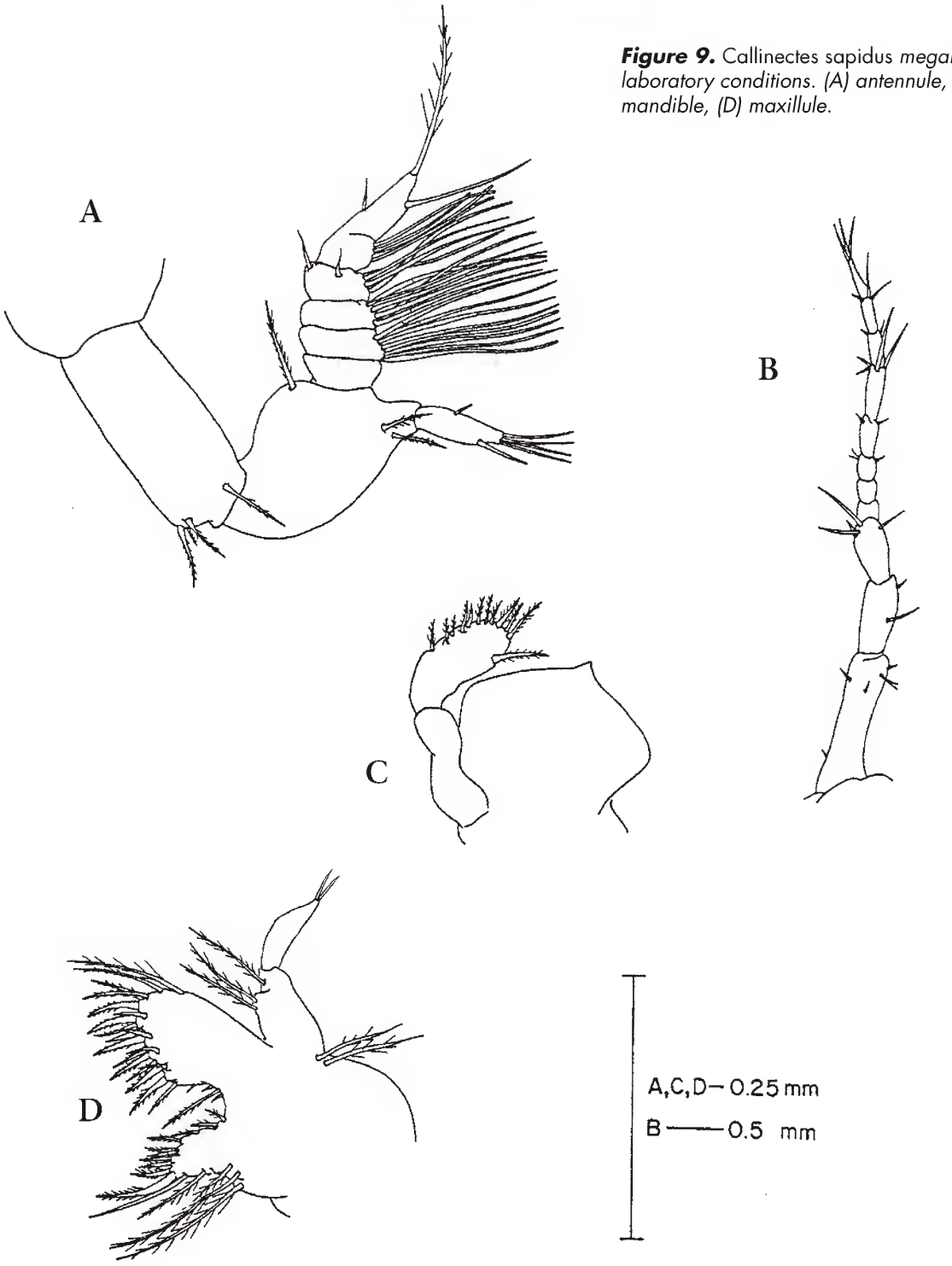

Figure 9. Callinectes sapidus *megalopa reared under laboratory conditions. (A) antennule, (B) antenna, (C) mandible, (D) maxillule.*

Zoea VII. Total length 2.62-3.34 mm (Figure 7)

Carapace (Figure 7A): similar to sixth zoea but larger, posterior ventral margin bearing 10-12 setae; chela bud well developed, "claw-like" with distinct articles, remaining appendage buds lengthened from sixth zoea. Rostral spine 0.77-1.14 mm, dorsal spine 0.75-1.50 mm, carapace spine width 1.22-1.67 mm, carapace spine length 2.33-3.24 mm.

Antennule and antenna (Figure 7B): antennule with large swelling at base, endopodite bud visible, aesthetascs arranged in 3 rows (4 plus 1 seta terminal, 4 to 6 subterminal and 4 to 5 basal). Endopodite bud of antenna equal to or slightly shorter in length to protopodite, swollen at base and showing evidence of articulation.

Maxillule (Figure 7C): endopodite unchanged from sixth zoea; basal endite of protopodite with 15 to 16 total plumose setae, coxal endite with 12 to 14 plumose setae.

Maxilla (Figure 7D): scaphognathite with 27 to 36 marginal plumose setae; endopodite unchanged from sixth zoea; basal endite of protopodite with 12 to15 total plumose setae, inner lobe of coxal endite with 5 plumose setae, outer lobe with 4 or 5 plumose setae.

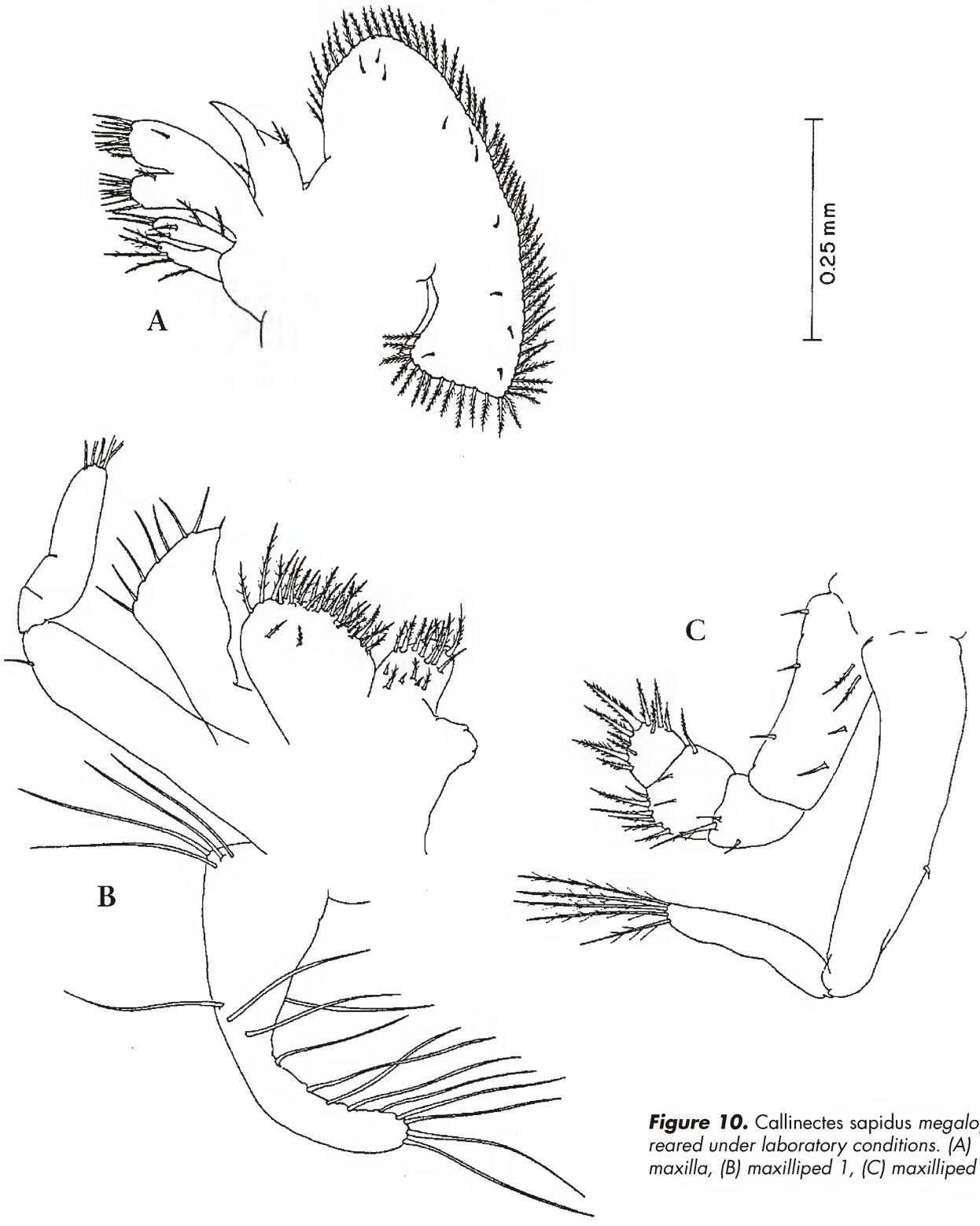

Figure 10. Callinectes sapidus *megalopa reared under laboratory conditions. (A) maxilla, (B) maxilliped 1, (C) maxilliped 2.*

Maxilliped 1 (Figure 7E): exopodite with 12 or 13 (rarely 11) terminal plumose setae; epipodite present as a T-shaped lobe bearing 2 setae on posterior margin; endopodite unchanged from sixth zoea.

Maxilliped 2 (Figure 7F): exopodite with 14 to 15 (rarely 13) terminal plumose setae; endopodite unchanged from sixth zoea.

Abdomen and telson (Figure 7A, G): pleopod buds lengthened from sixth zoea, clearly visible on segment 6. Inner medial margin of telson furca with 3 (rarely 4) small setae, remainder unchanged from sixth zoea.

Megalopa. Total length 2.62-3.80 mm (Figures 8-11)

Carapace and abdomen (Figure 8): carapace with pointed rostrum extending three-fourths length of antenna, rostral length 0.38-0.58 mm, carapace length 1.62-2.11 mm; pair of large spines project from the posterior ventral

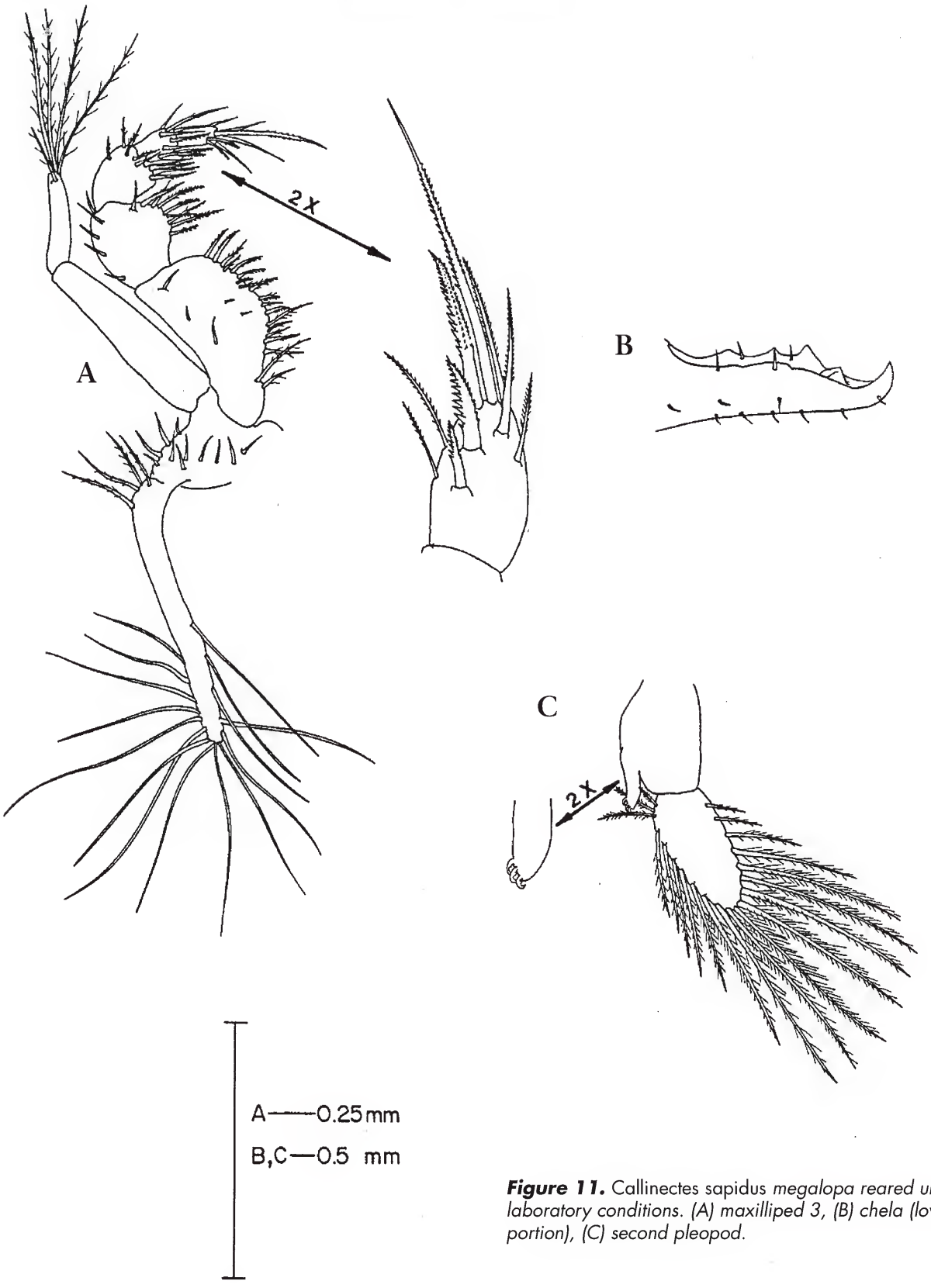

Figure 11. Callinectes sapidus *megalopa reared under laboratory conditions. (A) maxilliped 3, (B) chela (lower portion), (C) second pleopod.*

margin of cephalothorax; abdomen composed of 6 segments and telson, fifth segment with large lateral spines. Eyes stalked. Pigmentation pattern similar to that shown by Costlow and Bookhout (1959).

Antennule (Figure 9A): peduncle consisting of 3 articles, second article with 3 or 4 plumose setae on distal margin, third article with single medial seta and pair of distal setae; uniarticulate flagellum with 3 or 4 terminal setae and 2 subterminal; larger multiarticulated flagellum with 4 tiers of aesthetascs in arrangement of 8 or 9, 8 or 9, 8 or 9, 4; distal article with 1 terminal plumose seta and 2 subterminal setae.

Antenna (Figure 9B): consisting of 3 peduncle and 8 flagella articles, exact arrangement of setae variable, but often as shown.

Mandible (Figure 9C): palp with 2 articles, distal article with 11 to 12 spines, cutting edge smooth with medial "tooth".

Maxillule (Figure 9D): endopodite with proximal article bearing 3 or 4 plumose setae on outer distal half and 2 plumose setae at base near inner margin; terminal article bearing 1 or 2, usually 2, small, simple terminal setae; basal endite of protopodite with 20 to 23 plumose setae; coxal endite with 14 to 16 plumose setae of variable lengths.

Maxilla (Figure 10A): scaphognathite bearing 47 to 70 marginal plumose setae; endopodite lacking terminal setae; basal lobes of protopodite each with 8 to 9 terminal plumose setae and 1 subterminal seta; coxal lobes each with 5 to 6 total plumose setae.

Maxilliped 1 (Figure 10B): epipodite well developed with proximal cluster of 4 long simple setae and 13 to 14 distal simple setae; exopodite with 2 articles, bearing 5 long terminal setae (shown cut off) on distal article; endopodite with broad distal margin bearing 6 to 8 simple setae; basal lobe of protopodite with 23 to 25 marginal and 1 to 2 submarginal plumose setae, coxal lobe with 9 to 10 marginal and about 6 submarginal plumose setae.

Maxilliped 2 (Figure 10C): endopodite consisting of 4 articles, terminal article armed with 9 to 10 plumose setae, armature of remaining articles variable, but generally as shown; exopodite with 2 articles, terminal article with 5 to 6 plumose setae.

Maxilliped 3 (Figure 11A): endopodite consisting of 5 articles, setation variable but generally as shown, terminal article with several stout dentate setae; exopodite with 2 articles, distal article bearing 5 to 6 plumose setae, epipodite with cluster of 10 to 12 short setae and 4 long plumose setae on proximal portion, distal half with 15 to 17 long simple setae.

Chela (Figure 8A, 11B): well developed with prominent spine on basi-ishiopodite (not shown), distal process of propodus with four teeth in addition to terminal tooth.

Pleopods (Figure 11C): present on abdominal segments 2 to 6; endopodites developed on pleopods 1 to 4, bearing small curled setae on distal margin arranged as follows: first pleopod - 3 (rarely 4), second - 3, third - 3, fourth - 3; exopodites of pleopods 1 to 5 with long plumose setae arranged as follows: first pleopod - 20 to 22, second - 20 to 22, third - 20 to 22, fourth - 19 to 20, fifth - 10 to 11.

First Crab. (Figure 12A)

Carapace: carapace width 2.07-2.95 mm, two prominent lateral spines and 8 to 9 smaller spines (including outer margin of orbital socket), frontal margin slightly convex with small medial notch.

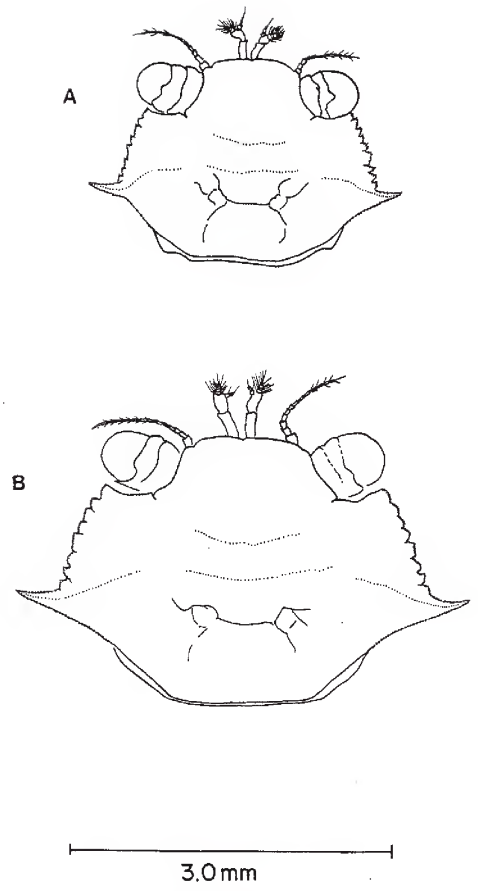

Figure 12. *Juvenile crabs of* Callinectes sapidus *reared under laboratory conditions. (A) first crab stage, (B) second crab stage.*

Second Crab (Figure 12B)

Carapace: carapace width 2.58-3.91 mm, similar to first crab except larger, medial notch less obvious.

Seasonal Variations in Laboratory Reared Larvae

Using developmental stages for which complete data sets were available (zoeal stages I-VII and the megalopal stage), the variation in total length, antennal length, dorsal spine, rostral spine, carapace spine length, and carapace spine width were summarized (range and mean) by season (Table 4). In all data sets examined, there were no notable differences observed between larvae obtained from different females within the same season or larvae at optimal and ambient temperatures within the same season. Differences between spring and summer/fall brood larvae were observed, but only in early stages (Table 4). The first, second and third zoeae from the spring brood were larger than corresponding summer/fall zoeae for all structures measured and ranges for spring and summer/fall did not overlap for first and second zoeae. Data sets on zoeal stages IV and V were incomplete; however, seasonal differences in measurements on all structures tended to be smaller in the summer/fall reared larvae although there was substantial overlap in the ranges. No seasonal differences were observed for the sixth and seventh zoeal stages and the megalopal stage. First

TABLE 4. *Comparison of spring and summer/fall reared blue crab zoeae, megalopae and first crabs. Measurements (mm) of range and means (in parentheses) on larval characters were calculated from combined data on all females and experimental temperatures. TL = Total length (carapace width for first crab); AL = Antennal length; DS = Dorsal spine; RS = Rostral spine (rostral length for megalopa); CSL = Carapace spine length (carapace length for megalopa); CSW = Carapace spine width.*

Stage	Season	TL	AL	DS	RS	CSL	CSW
1st zoea	Spring	1.10-1.25 (1.19)		0.43-0.48 (0.47)	0.25-0.30 (0.29)	1.00-1.17 (1.09)	0.58-0.67 (0.63)
	Summer/Fall	0.90-0.99 (0.94)		0.30-0.35 (0.33)	0.20-0.23 (0.21)	0.79-0.87 (0.83)	0.45-0.52 (0.49)
2nd zoea	Spring	1.22-1.45 (1.35)		0.43-0.55 (0.49)	0.32-0.42 (0.37)	1.14-1.30 (1.20)	0.62-0.73 (0.67)
	Summer/Fall	0.94-1.14 (1.07)		0.30-0.40 (0.35)	0.22-0.32 (0.29)	0.80-0.99 (0.91)	0.47-0.58 (0.52)
3rd zoea	Spring	1.32-1.80 (1.59)		0.45-0.67 (0.56)	0.32-0.53 (0.44)	1.17-1.60 (1.41)	0.68-0.87 (0.77)
	Summer/Fall	1.12-1.47 (1.27)		0.32-0.55 (0.43)	0.32-0.40 (0.35)	1.02-1.40 (1.10)	0.58-0.75 (0.62)
4th zoea	Spring	1.65-2.15 (1.92)		0.53-0.84 (0.68)	0.45-0.67 (0.56)	1.47-2.04 (1.72)	0.77-1.10 (0.90)
	Summer/Fall	1.50-1.98 (1.69)		0.43-0.58 (0.60)	0.35-0.58 (0.48)	1.19-1.84 (1.49)	0.67-1.00 (0.81)
5th zoea	Spring	1.70-2.40 (2.21)		0.58-0.95 (0.75)	0.42-0.78 (0.66)	1.70-2.30 (2.02)	0.89-1.17 (1.00)
	Summer/Fall	1.87-2.27 (2.11)		0.58-0.97 (0.77)	0.53-0.75 (0.63)	1.60-2.20 (1.92)	0.85-1.14 (1.00)
6th zoea	Spring	2.33-2.84 (2.50)		0.80-1.12 (0.95)	0.67-0.92 (0.80)	2.04-2.60 (2.31)	1.07-1.27 (1.18)
	Summer/Fall	2.24-2.66 (2.50)		0.75-1.14 (0.96)	0.67-0.89 (0.77)	1.98-2.50 (2.29)	1.04-1.34 (1.19)
7th zoea	Spring	2.62-3.28 (2.99)		0.75-1.44 (1.14)	0.87-1.10 (0.98)	2.33-3.18 (2.78)	1.23-1.50 (1.39)
	Summer/Fall	2.66-3.34 (3.06)		1.00-1.50 (1.19)	0.77-1.14 (0.95)	2.40-3.24 (2.81)	1.22-1.67 (1.40)
Megalopa	Spring	2.75-3.60 (3.27)	0.72-1.13 (0.99)		0.38-0.57 (0.49)	1.62-2.07 (1.89)	
	Summer/Fall	2.62-3.80 (3.34)	0.96-1.09 (1.01)		0.47-0.58 (0.50)	1.62-2.11 (1.94)	
First Crab	Spring	2.07-2.91 (2.67)					
	Summer/Fall	2.27-2.95 (2.63)					

TABLE 5. *Characteristics of* C. sapidus *megalopae collected from the plankton and reared in the laboratory along with measurements of first crabs reared from these megalopae. Mean values (mm) in parentheses obtained from measurements on 10 megalopae and 20 crabs.*

Character	Total Carapace Length (mm)	Rostral Length (mm)	Antennal length (mm)	Carapace width (mm)
Megalopae				
Spring	2.06-2.24 (2.16)	—	1.14-1.20 (1.16)	
Fall	1.82-1.96 (1.89)	0.44-0.48 (0.47)	1.02-1.08 (1.04)	
First crabs				
Spring				3.0-3.2 (3.1)
Fall				2.4-2.7 (2.6)

crabs reared from spring and summer/fall larvae were also similar in size.

Seasonal Variations in Wild Stock Megalopae and First Crabs

Spring megalopae were always larger than fall megalopae with mean total carapace lengths of 2.16 mm and 1.89 mm, respectively (Table 5) and the seasonal ranges in size did not overlap. Similar differences were observed for mean measurements of antennal length (spring – 1.16 mm; fall – 1.04 mm). Spring megalopae also tended to have more setae on the scaphognathite of the maxilla and epipodite of the third maxilliped (Table 6). Few seasonal differences were observed in the number of plumose setae on the exopods of the pleopods; however, the number of hooked setae on the endopods was different between seasons. Spring megalopae normally had four (rarely three) hooked setae on pleopods 1 and 2 while pleopods 3 and 4 had three (rarely four) such setae. In contrast, fall megalopae had three (occasionally four) hooked setae on pleopods 1 and 2 and always three on pleopods 3 and 4. First crabs followed the same seasonal size trends observed for megalopae (spring, mean carapace width – 3.1 mm; fall, mean carapace width – 2.6 mm with no overlap in size ranges; Table 5).

Discussion

Morphological Development

Seven zoeal and one megalopal stage were identified; eighth and ninth zoeal stages were rarely found. Although larvae described here resemble those originally described by Costlow and Bookhout (1959) in most major features, a number of differences in overall size and setation of appendages were noted.

Throughout the entire larval series, measurements on a number of larval structures from individual stages were consistently larger than the corresponding measurements reported by Bookhout and Costlow (1977). Differences between larvae from the nGOM (present study) and the original description of those from the Atlantic coast (Costlow and Bookhout 1959, Bookhout and Costlow 1977) are summarized below.

Carapace: For all zoeal stages of nGOM larvae, a minute seta was observed just below and lateral to the base of the dorsal spine. A single seta was found on the anterior margin of the carapace just above the eyes on zoeal stages II to VII. These setae were not found by Costlow and Bookhout (1959).

Antennules: The numbers of terminal aesthetascs found from nGOM larvae were fairly consistent with Atlantic coast larvae. The addition of a third row of aesthetascs occurred in stage VII zoeae in the present study (Figure 7), whereas Bookhout and Costlow (1977) reported the third row only from stage VIII zoeae. Terminal "hooked setae" were not found in nGOM larvae. All terminal setae appeared relatively straight. One or two terminal "hair-like" setae were found on nGOM specimens, whereas, Bookhout and Costlow (1977) reported two to three "hair-like setae" from Atlantic specimens. For the megalopae, Bookhout and Costlow (1959) described and illustrated the terminal article of the flagellum with

TABLE 6. *Characteristics of* C. sapidus *megalopae collected from the plankton and reared in the laboratory. Mean values in parentheses obtained from measurements on 10 megalopae.*

Character	Spring	Fall
Scaphognathite of maxilla		
Marginal setae	61-69 (65)	59-66 (62)
Submarginal setae	10-14 (11)	11-13 (12)
Epipodite of 3rd maxilliped		
Proximal setae	4-5 (4)	4
Distal setae	14-20 (17)	12-18 (16)
Plumose setae on exopods of pleopods		
Pleopod 1	22-24 (22)	22-24 (23)
Pleopod 2	21-23 (22)	21-23 (23)
Pleopod 3	20-23 (21)	20-23 (22)
Pleopod 4	19-21 (20)	18-21 (19)
Pleopod 5	11-12 (11)	11-12 (11)
Hooked spines on endopods of pleopods		
Pleopod 1	3-4 (4)	3-4 (3)
Pleopod 2	3-4 (4)	3-4 (3)
Pleopod 3	3-4 (3)	3
Pleopod 4	3-4 (3)	3

a simple terminal seta; however, for the nGOM specimens examined the terminal seta is plumose (Figure 9A), though the plumose condition may need confirmation at high magnification.

Mouthparts: Variability in mouthparts of larvae obtained in the present study complicates detailed comparison with Atlantic larval descriptions. In general, setation on the maxillule and maxilla of early stage zoeae as reported by Bookhout and Costlow (1977) was similar to nGOM larvae. In later stages, however, nGOM larvae tended to have a greater number of total spines and setae. This may be the result of fewer larval stages produced in the present study as compared to previous studies. One obvious difference between Atlantic and nGOM larvae is in the setation of the scaphognathite in early stage larvae. Costlow and Bookhout (1959) illustrated the distal margin of the scaphognathite of zoeal stages I and II as bearing 2 plumose setae. In the present study, all larvae examined had one and 3 such distal setae on zoeal stages I and II, respectively (Figures 1 and 2). For megalopae, Costlow and Bookhout (1959) describe the terminal article of the maxillule endopodite as bearing 4 long, plumose setae (two terminal, two subterminal), whereas, the nGOM specimens examined had only 2 terminal short, simple setae. Also, the 4 plumose terminal setae on the Atlantic specimens appear distinctly larger than the 2 simple terminal setae in the nGOM specimens.

Telson: A minute seta (visible only under high magnification) located just posterior to the insertion of a large lateral seta was observed in all zoeae examined in the present study (Figures 1-7). This minute seta was not reported in previous studies.

Seasonal Variations in Morphology

In the present study, early stage zoeae (I, II and III) reared from spring females were larger than summer/fall brood larvae. Differences in total size between spring and summer/fall larvae among intermediate and later stage zoeae were less apparent. Virtually no seasonal differences were observed in size of laboratory reared megalopae and first crabs. In contrast, seasonal differences were observed in megalopae and first crabs reared from the plankton. Wild stock megalopae and first crabs obtained in the spring were substantially larger than fall megalopae and first crabs.

The inability to reproduce seasonal variations in morphology through the entire larval series in the laboratory, as it occurs in the plankton, may be the result of factors influencing development other than temperature. Because of their large initial size, spring brood zoeae when reared at a static optimal temperature (25°C) and provided with excess food may have been able to feed more efficiently than larvae from other seasonal/temperature combinations and thus developed to megalopae in less time and with fewer total molts. Spring larvae initially reared at l6.0°C did not molt until culture temperature had increased to 19.0°C (18 d later). As the culture temperature continued to increase, at a rate of about 1.0°C/week, molting frequency also increased. Again, their large initial size and resulting increased feeding ability allowed most of these larvae to complete their development to megalopae in 6 molts. In contrast, summer/fall brood larvae reared at 25.0°C or 30.0°C molted more frequently than spring larvae; however, because of their smaller initial size and subsequent reduced ability to capture food, these larvae usually required 7 or more molts to complete their development. The tendency for greater number of larval stages and longer duration of development (at 25.0°C) may be the mechanism by which summer/fall larvae eventually equaled the size of spring larvae in laboratory culture. Assuming that larval growth is under the principal control of nutrition, all culture series would have the same growth potential. Other studies have found that the effects of quality and quantity of food on decapod larval development have been significant (Broad 1957, Chamberlain 1961, Welch and Sulkin 1974, Sulkin 1975, 1978, Terwilliger and Dumler 2001) and may be the principal factor controlling larval growth. Variation in size of megalopae and first crabs obtained from the plankton may also be related to seasonal trends in food availability rather than temperature. Investigations on trophic relationships of larvae in the plankton may provide further insights into seasonal variability of *C. sapidus* larvae.

Eco-phenotypic seasonal size differences have also been found to exist in other life stages of blue crab. Jacobs et al. (2003) found the eggs of *C. sapidus* were larger in the spring and similar results were found by Dennis (2008). Furthermore, Perry et al. (2007) found that spring females were larger than summer/fall females during a fishery-dependent survey of blue crab entering the trap fishery in Mississippi. Similar trends were noted in long-term monitoring data for adult females in Mississippi (GCRL, unpublished data[1]) and for legal-size crabs from Louisiana (LDWF, unpublished data[2]).

Although the number of zoeal stages required to reach the megalopal stage in this study varied from 6 to 9, in most cases (51%) 7 zoeal stages were observed. Larvae spawned in spring generally completed development to megalopae in fewer molts than did larvae spawned in summer/fall. In cases where development was completed in only 6 molts, zoeae with combined morphological characteristics from 2 stages were usually observed. This occurred most frequently in spring brood larvae between zoeal stages V and VII. Costlow (1965) reported that most (63%) of the

(1) GCRL. Unpublished data. Fisheries assessment and monitoring program, 1973-2005. The University of Southern Mississippi, Gulf Coast Research Laboratory, Ocean Springs, Mississippi.

(2) LDWF. Unpublished data. Fisheries independent long-term monitoring, 1967-2005. Louisiana Department of Wildlife & Fisheries, Baton Rouge, Louisiana.

larvae of C. sapidus reared at 25.0°C passed through 7 zoeal stages before reaching the megalopal stage; 21% had 6 zoeal stages and 16% passed through 8 zoeal stages. Morphological variability was observed in 40 to 63% of these larvae. Costlow (1965) also found that zoeal stages I to IV showed little morphological variability while stages V to VII commonly differed. The most frequently encountered variation was the occurrence of "combined stage" zoeae V/VI and VI/VII. Similar results were obtained in the present study.

In conclusion, the results of this study indicate that: (1) morphological differences in size and setation exist between *C. sapidus* larvae reared from the nGOM stocks and published descriptions of larvae reared from Atlantic stocks; (2) laboratory reared spring brood larvae were larger than summer/fall brood larvae among early zoeal stages (I, II and III); later stage zoeae, megalopae and first crabs were similar in size between seasons; (3) fewer larval stages occurred in the spring than in the summer/fall; and (4) megalopae and first crabs reared from the plankton were larger in the spring, which could not be reproduced in the rearing experiment.

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A Comparison of Fish Populations in Shallow Coastal Lagoons with Contrasting Shoalgrass (*Halodule wrightii*) Cover in the Northcentral Gulf of Mexico

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SHORT COMMUNICATION

A COMPARISON OF FISH POPULATIONS IN SHALLOW COASTAL LAGOONS WITH CONTRASTING SHOALGRASS (*HALODULE WRIGHTII*) COVER IN THE NORTHCENTRAL GULF OF MEXICO

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Introduction

It is widely accepted that seagrass meadows provide abundant food and structure for a variety of organisms (see reviews by Hemminga and Duarte 2000, Williams and Heck 2001). The seagrasses themselves, epiphytes and macroalgae constitute a diverse array of food resources for first-order consumers, which in turn fuels a complex and rich food web in seagrass meadows (Williams and Heck 2001). Enhanced structure in seagrass meadows is mainly provided by the canopy of leaf blades and sheaths, drifting macroalgae and bulky epiphytes (Orth et al. 1984).

Large seagrass declines, mostly due to anthropogenic impacts, have been reported worldwide (i.e., Orth et al. 2006), and shoalgrass (*Halodule wrightii*), which is common in subtropical and tropical Atlantic waters (Den Hartog 1970), is no exception. For instance, Pulich and White (1991) documented that in lower Galveston Bay shoalgrass declined steadily since the 1950's and disappeared completely in the 1980's mainly due to urban development, wastewater discharges, chemical spills and dredging. Similarly, Quammen and Onuf (1993) estimated a >330 km^2 reduction in shoalgrass cover in lower Laguna Madre from 1965 to 1988 as a result of increased turbidity caused by dredging. Shoalgrass beds continue to decline in a number of locations in the Gulf of Mexico (GOM) (Hall et al. 1999).

A number of studies have examined the effects of reduced seagrass cover on local fish populations (e.g., Heck et al. 1989, Ferrell and Bell 1991, Hughes et al. 2002 and more), but few of those studies have focused on shoalgrass (e.g., Tolan et al. 1997, Rydene and Matheson 2003). We present a preliminary comparison of fish populations in three shallow coastal lagoons in the northcentral GOM that have varying levels of shoalgrass cover. Namely, we compare (1) abundances of individual species and the entire fish population, (2) fish population diversity, and (3) length-frequency distributions of the most abundant species.

Materials and Methods

The study was done in three shallow (mean depth < 1m) coastal lagoons situated at the southern end of Perdido Bay (FL, USA) in the northcentral GOM. Water-column temperature, salinity and dissolved oxygen concentrations were similar in the three lagoons. In contrast, shoalgrass cover differs notably among the lagoons. State Park is the most vegetated lagoon, Kee's Bayou has little shoalgrass, and Gongora has no shoalgrass. Other than shoalgrass, the bottom of the lagoons is characterized by open sediment consisting of sand and mud. A full description of the lagoons is provided by Stutes et al. (2007).

Fish were collected using a 6.0 m x 1.2 m bag seine with 3-mm mesh. Seining is an adequate method for the capture of the small fish, including juveniles of larger species, that typically predominate in shallow coastal embayments such as the three lagoons studied here (Connolly 1994, Rozas and Minello 1997). To examine the fish populations before and after the fall migration that many fish in these shallow embayments exhibit with the arrival of the first cold fronts (Stoner 1983, Middaugh and Hemmer 1987a), we sampled in late summer (i.e., pre-migration samples, September 12-18, 2000) and after several cold fronts had moved through the area and water temperature in the lagoons had decreased significantly (i.e., post-migration samples, October 23-25, 2000).

In each lagoon on each sampling date, we seined sixteen, 20 m transects. Transect selection was haphazard. We estimated the area covered by shoalgrass at 1m intervals along each transect. Each interval was considered to be covered by shoalgrass if cover was greater than 50%. The fraction of the entire transect covered with shoalgrass was the number of intervals with >50% cover divided by 20, and a grand average was calculated for all the transects in the lagoon. To reduce personal bias, two individuals (JC and GAM) did all the seining, and a third individual (JPS) made all estimates of shoalgrass cover.

All fish were taken to the lab, where they were counted and identified. Diversity was calculated using the reciprocal of the Simpson index (1/D) after pooling all the seines on each sampling date at each lagoon to increase the robust-

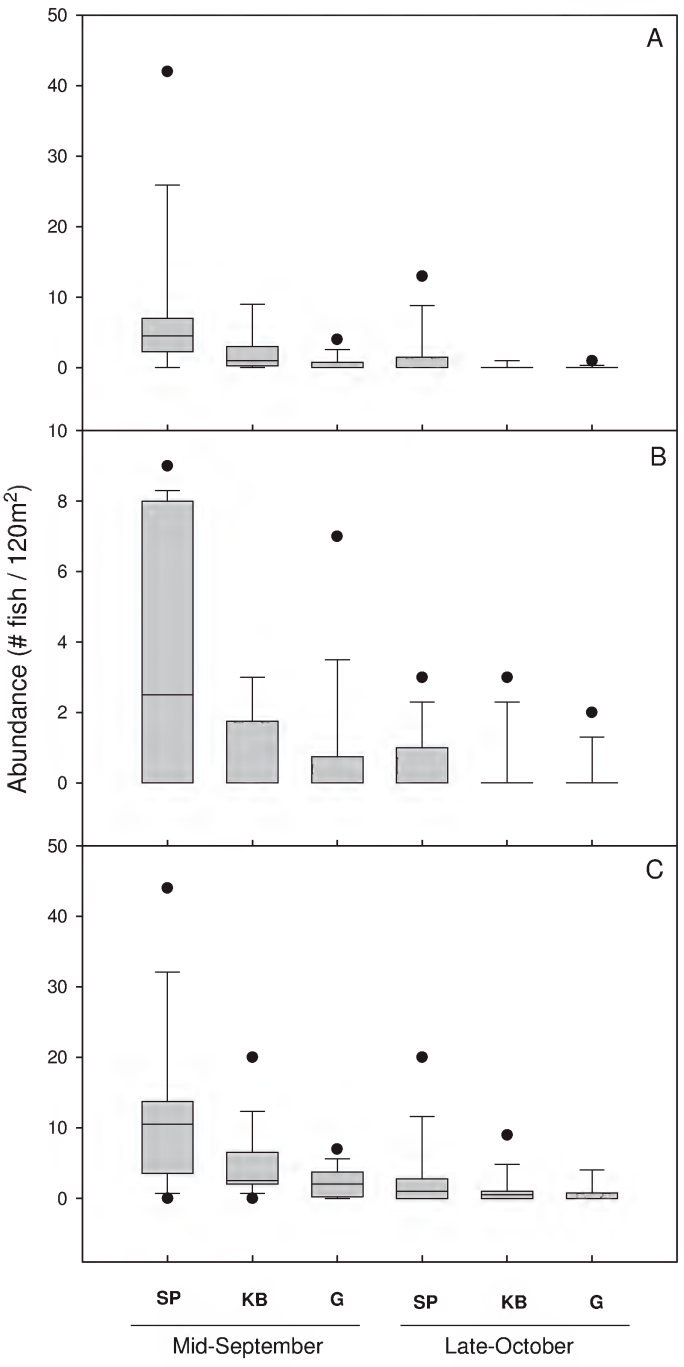

Figure 1. *Box plots of abundance of (A) tidewater silversides, (B) juvenile pinfish, and (C) the fish population in State Park (SP), Kee's Bayou (KB) and Gongora (G) in mid September and late October. Boxes encompass the 25% and 75% quartiles, and the central line represents the median, for the sixteen seines in each lagoon on each date. Bars encompass the range of values between (1) the 25% quartile minus 1.5 times the difference between the quartiles 75% and 25% and (2) the 75% quartile plus 1.5 times the difference between the quartiles 75% and 25%. Circles represent values outside these limits.*

ness of the diversity values. We chose D over other metrics of diversity because we found large differences in total fish abundance across the lagoons, and D is known to be robust against those differences (Magurran 2004). We also measured the standard length (SL, mm) of all tidewater silversides (*Menidia peninsulae)* and juvenile pinfish (*Lagodon rhomboides*). We did not capture enough individuals of other species to make comparisons of fish length among lagoons.

Differences in abundances of individual species and of the entire fish population across the three lagoons and between the two sampling dates were analyzed with the Scheirer-Ray-Hare extension of Kruskal-Wallis, a non-parametric test in lieu of two-way ANOVA, due to the non-compliance of the data with the assumptions of ANOVA (Sokal and Rohlf 1995). Post-hoc comparisons between lagoons were done with the non-parametric Q test (Zar 1998). Differences in fish diversity across lagoons were analyzed with one-way ANOVA for each sampling date, and post-hoc comparisons were conducted with Tukey tests (Magurran 2004) after calculating the variance of D as explained in Lande (1996). Differences in the SL of tidewater silversides and juvenile pinfish among lagoons were analyzed only in mid September, because too few individuals of those species were captured in Kee's Bayou and Gongora in late October. Differences in fish SL were analyzed with the non parametric Kruskal Wallis and post-hoc Q tests due to the non compliance of the data with the assumptions of ANOVA. All tests were performed using SigmaStat 8 and considered significant if $p < 0.05$, marginally significant if $0.05 \leq p < 0.10$, and non-significant if $p \geq 0.10$.

Results

The areas swept with the seine had mean (± se) shoalgrass covers of 17.0 (± 0.9), 2.0 (± 0.3) and 0 % in State Park, Kee's Bayou and Gongora, respectively, which reflects well the differences in total shoalgrass cover (i.e., cover for the entire lagoon) among the three lagoons (Stutes et al. 2007).

We captured twenty fish species (Table 1). The two most abundant species in all three lagoons in mid September were tidewater silversides and juvenile pinfish. In late October, these species continued to be the most abundant in State Park, they co-dominated along with bay anchovy (*Anchoa mitchilli*) and spot (*Leiostomus xanthurus*) in Kee's Bayou, and spotfin mojarra (*Eucinostomus argenteus*) and juvenile pinfish were the dominant species in Gongora.

Abundances of tidewater silversides and juvenile pinfish were higher in mid September than in late October (Figures 1A and B; Scheirer-Ray-Hare test for each species, $p < 0.05$). Abundances of these two species were also higher in State Park than in Gongora (Q test between the two lagoons for each species, $p < 0.05$), and those differences persisted on both sampling dates as indicated by the lack of a significant interaction between lagoon and time (Scheirer-Ray-Hare test for each species, $p \geq 0.10$). Comparisons of total fish abundance yielded similar results due to the dominance or co-dominance by tidewater silversides and juvenile pinfish in the lagoons studied (Figure 1C). Total fish abundance was higher in mid September than in late October, and it was higher in State Park than in Gongora regardless of sam-

TABLE 1. *Fish captured in three lagoons in the north-central Gulf of Mexico. Numbers with no parentheses are the total number of individuals of each species captured in sixteen seine hauls per lagoon per sampling period, and numbers in parentheses indicate the percentage of the total number of fish captured per lagoon per sampling period.*

	State Park		Kee's Bayou		Gongora	
	Mid-September	Late-October	Mid-September	Late-October	Mid-September	Late-October
Anchoa mitchilli (bay anchovy)			1 (1.4)	6 (30.0)	1 (2.9)	
Dorosoma petenense (threadfin shad)						1 (9.1)
Harengula jaguana (scaled sardine)	4 (2.2)				4 (11.4)	
Anguilla rostrata (American eel)					1 (2.9)	
Ariopsis felis (hardhead catfish)			1 (1.4)			
Mugil cephalus (striped mullet)			5 (6.9)	2 (10.0)	5 (14.3)	
Menidia peninsulae (tidewater silverside)	117 (63.2)	24 (54.5)	39 (54.2)	3 (15)	9 (25.7)	1 (9.1)
Adinia xenica (diamond killfish)		4 (9.1)			1 (2.9)	
Fundulus grandis (Gulf killifish)		1 (2.3)				
Poecilia latipinna (sailfin molly)		2 (4.5)			1 (2.9)	
Syngnathus scovelli (Gulf pipefish)		1 (2.3)				
Chloroscombrus chrysurus (Atlantic bumper)	3 (1.6)					
Oligoplites saurus (leatherjack)			6 (8.3)			
Eucinostomus argenteus (spotfin mojarra)			5 (6.9)	1 (5.0)	2 (5.7)	4 (36.4)
Eucinostomus gula (silver jenny)	1 (0.5)		2 (2.8)			
Archosargus probatocephalus (sheepshead)		1 (2.3)				
Lagodon rhomboides (pinfish)	59 (31.9)	9 (20.5)	11 (15.3)	5 (25.0)	11 (31.4)	4 (36.4)
Bairdiella chrysoura (silver perch)						1 (9.1)
Leiostomus xanthurus (spot)			2 (2.8)	3 (15.0)		
Achirus lineatus (lined sole)	1 (0.5)	2 (4.5)				
Total	**185**	**44**	**72**	**20**	**35**	**11**

pling period.

Contrary to abundance, diversity increased from State Park (1/D = 2) to Kee's Bayou (1/D = 3.1) to Gongora (1/D = 5.5) in mid September (all three Tukey tests, $p < 0.05$). Diversity was lower in State Park (1/D = 3) than in Kee's Bayou (1/D = 5.9) in late October (Tukey test, $p < 0.05$).

In mid September tidewater silverside were larger (mean SL ± se) in Gongora (53.6 ± 0.7 mm) than in Kee's Bayou (51.0 ± 0.5 mm) (Q test, $p < 0.05$), and marginally larger in Gongora than in State Park (51.6 ± 0.3 mm) (Q test, $p = 0.07$). Juvenile pinfish were larger in Gongora (68.9 ± 1.6 mm) than in State Park (54.6 ± 1.7 mm) (Q test, $p < 0.05$).

Discussion

Our results are only based on two sampling dates. In addition, despite encompassing a gradient in shoalgrass cover (Stutes et al. 2007), we only sampled three lagoons. Finally our seine was rather short and some fast-swimming fish could have escaped from it. Thus, our results can only be viewed as preliminary. At any rate, our findings reveal a number of significant differences among the lagoons and suggest important effects of contrasting shoalgrass cover on the fish populations.

Abundance of tidewater silversides and juvenile pinfish decreased across lagoons as the shoalgrass cover in the lagoon decreased. This may be due to selection of seagrass beds over bare sediment as nursery habitat, most likely due to enhanced physical structure (which in turn provides more protection against predators and wave energy) and food availability (Stoner 1983, Jordan et al. 1996, Tolan et al. 1997). Adult tidewater silversides spawn preferentially on seagrass leaves and attached macroalgae upon their return to shallow embayments in late winter/early spring (Middaugh and Hemmer 1987b). The young-of-the-year grow fast and most of them reach adult size (60-70 mm SL) by the end of the summer (Lucas 1982, Middaugh and Hemmer 1987b). Pinfish spawn offshore and juveniles recruit preferentially to seagrass beds in late winter/early spring, where they grow fast to reach 90-100 mm by the end of the summer (Hoss 1974, Spitzer et al. 2000, Nelson 2002). Measurements of abundance of the main prey for juvenile pinfish (i.e., amphipods, isopods and shrimp; Stoner 1982) in the three lagoons on September 5, 2000 indicate that food availability

for juvenile pinfish decreases across the lagoons as shoalgrass covers decreases. This may also be the case for tidewater silversides, since most of their prey (i.e., suspended organic matter, their own larvae -for adults only-, and some benthic prey such as amphipods; McMullen and Middaugh 1985) is more abundant within the canopy of seagrass beds than in open water (Tolan et al. 1997, Gacia et al. 2002).

Abundance of tidewater silversides and juvenile pinfish also decreased from mid September to late October, which was likely due to migration offshore. In the fall, as water temperature starts decreasing, most tidewater silversides and juvenile pinfish migrate from shallow embayments to deeper coastal waters, although a few overwinter in shallow embayments (Hoss 1974, Lucas 1982, Middaugh and Hemmer 1987a). Interestingly, the differences in abundance across the three lagoons persisted in late October, suggesting that a larger population of tidewater silversides and juvenile pinfish overwinter in the lagoon with the most shoalgrass.

Our finding that the mean size of tidewater silversides and juvenile pinfish increases as shoalgrass cover in the lagoon decreases suggests that shoalgrass beds offer effective protection against predators for small individuals of these two species. In accordance with these results, other comparisons have found that tidewater silversides sampled in seagrass-vegetated sites tended to be smaller than those sampled in areas of bare sediment (Tolan et al. 1997).

Because tidewater silversides and juvenile pinfish dominated or co-dominated the fish populations studied, differences in total fish abundance among the lagoons and between the two sampling dates mimicked those in the abundances of the two species. Total fish abundance was lower in late October than in mid September, and it decreased across lagoons as shoalgrass cover in the lagoon decreased regardless of the date considered. Interestingly, diversity showed an opposite tendency, with diversity increasing across the lagoons as shoalgrass cover in the lagoon decreased. This result emerges from a parallel reduction in the extent of dominance by tidewater silversides and juvenile pinfish as shoalgrass cover in the lagoon decreases, which renders the distribution of species relative abundance more even and leads to higher diversity as measured with the Simpon index. Indeed, tidewater silversides and juvenile pinfish accounted for 95, 69 and 57% of all the fish captured in State Park, Kee's Bayou and Gongora in mid September, and for 75, 40 and 45% in late Octoter.

Our results are consistent with previous reports that seagrass declines are deleterious for fish species that select seagrass beds as preferred habitat, and that the effect on the total local fish population will depend on how numerous seagrass-associated species are in relation to the other species in the population (e.g., Heck et al. 1989, Hughes et al. 2002, Vanderklift and Jacoby 2003). These results also suggest that losses of shoalgrass cover in shallow coastal lagoons may result in lower abundance of tidewater silversides and juvenile pinfish, which could entail reduced prey availability for the many predators than feed on these species as they migrate to deeper waters in the fall (Lucas 1982, Jordan et al. 1996). At any rate, our results are based on limited effort and sample size. Furthermore, the seine employed was rather short, which could lead to underestimating the abundance of some species. As such, our results can only be regarded as preliminary. For instance, previous comparisons have also found reduced tidewater silverside abundance in sites with less seagrass (Tolan et al. 1997), but other comparisons have only found a weak association between the abundance of tidewater silversides and seagrass cover (Rydene and Matheson 2003). Clearly, our findings need to be confirmed with longer, more complete studies.

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SHORT COMMUNICATION

FIRST RECORDS OF THE SEAGRASS PARASITE *PLASMODIOPHORA DIPLANTHERAE* FROM THE NORTHCENTRAL GULF OF MEXICO

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Introduction

The parasite *Plasmodiophora diplantherae* is a causal agent of enlarged shoot galls in seagrasses. Ferdinandsen and Winge (1914) described the genus *Ostenfeldia* for a plasmodiophoraceous organism in shoot galls of *Halodule wrightii* (as *Diplanthera wrightii*) collected from St. Croix, West Indies (now U.S. Virgin Islands). After re-examination of this material by Cook (1933), the parasite was renamed *Plasmodiophora diplantherae* (Ferd. & Winge) Ivimey Cook. Den Hartog (1965) re-examined additional herbaria specimens collected throughout the world and concluded *P. diplantherae* was specific to the host genus *Halodule*.

Traditionally studied with the fungi, members of the genus *Plasmodiophora* are flagellate protists (Patterson 1999) in the Phytomyxea (Eukaryotes, phylum Cercozoa) (Cavalier-Smith 1998). Informally called plasmodiophorids (Braselton 1995), these organisms are obligate endobionts in a diverse group of terrestrial, marine and freshwater hosts including higher plants, algae and oomycetes. Eleven genera are currently recognized, containing 36 species (Maier et al. 2000). Plasmodiophorids are characterized by a multinucleate vegetative structure called a plasmodium, which divides at maturity to form either (1) sporangia that produce biflagellate, motile zoospores, or (2) resting spores which are released when the host cell breaks (Karling 1968). Many plasmodiophorids induce cell hypertrophy in their hosts via cell enlargement (den Hartog 1989). In vascular plant hosts, however, the parasite is restricted to the inner cortex and thus plant growth can continue (den Hartog 1965). *Plasmodiophora diplantherae* causes enlarged internodes (galls) in host shoots which contain rust brown, smooth-walled resting spores 4-4.5 µm in diameter (Karling 1968).

Plasmodiophora diplantherae is known to occur throughout the pantropical distribution of its host, the seagrass genus *Halodule*. However, records in the subtropical region are limited to Tampa Bay, FL where it was detected once during an examination of herbarium specimens of *H. beaudettei* collected in December 1951 (den Hartog 1965) and to Fort Pierce, FL where it was collected once infecting *H. wrightii* (Braselton and Short 1985). This communication represents the first report of this parasite from Mississippi and Louisiana in the northcentral Gulf of Mexico (GOM).

Materials and Methods

During an investigation of seagrass roots in the northcentral GOM, seagrass plants were collected from three seagrass bed sites (Figure 1): Pointe aux Pines, AL (22 August 2006, site 1); Grand Bay National Estuarine Research Reserve (NERR), MS (9 September 2006, site 2); and Chandeleur Islands, LA (27 September 2006, site 3). Additional *H. wrightii* specimens were also collected from Horn Island, MS (13 October 2006, site 4) and from Chandeleur Islands, LA (19 June 2008, site 3).

Core samples (15 cm x 30 cm) containing seagrass roots and surrounding marine sediment were taken at three randomly selected points along two 25 m transects 100 m apart at sites 1, 2 and 3. Salinity (ppt), pH, dissolved oxygen con-

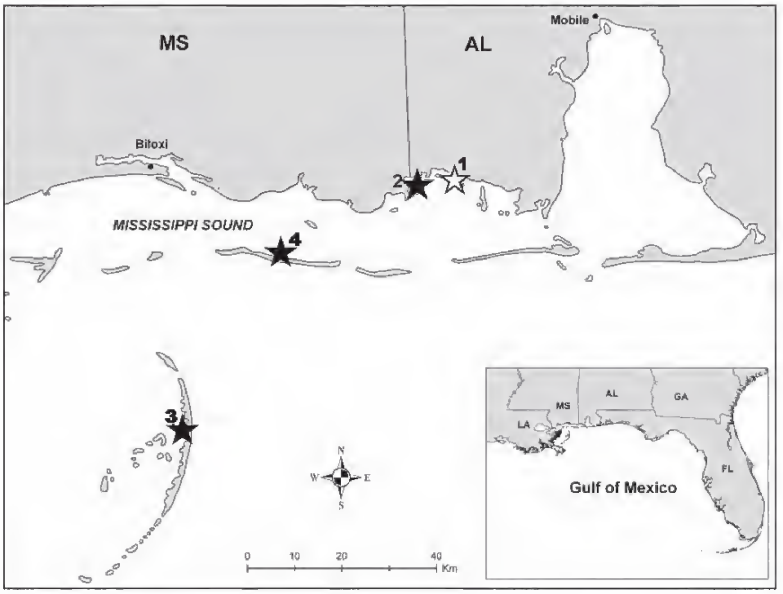

Figure 1. *Location of seagrass collecting sites within the northcentral Gulf of Mexico. Site 1 = Pointe aux Pines, AL; Site 2 = Grand Bay National Estuarine Research Reserve, MS; Site 3 = Chandeleur Islands, LA; Site 4 = Horn Island, MS. Black stars indicate sites at which* Halodule wrightii *parasite* Plasmodiophora diplantherae *was present.*

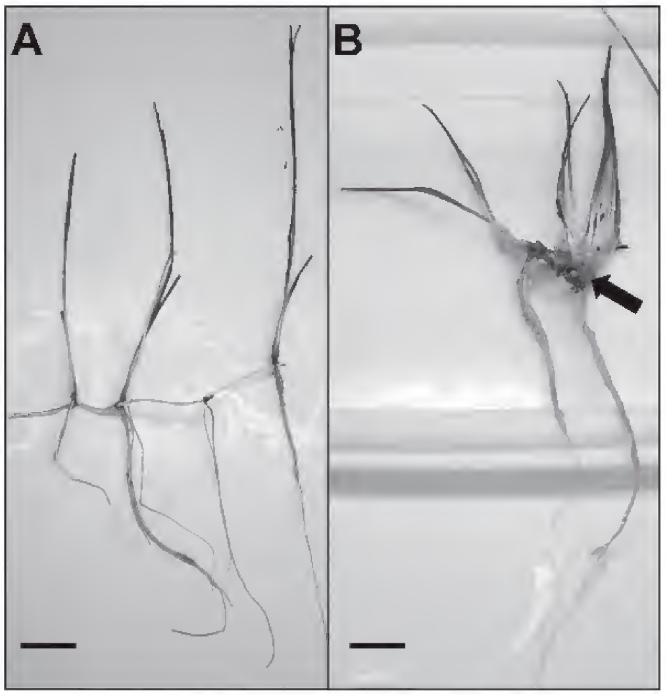

Figure 2. *A. Healthy* Halodule wrightii *plant. B.* Halodule wrightii *infected with* Plasmodiophora diplantherae, *causing galls at internodes (arrow). Scale bar = 2 cm.*

tent (%), and water temperature (°C) were recorded for each transect and water depth (m) was recorded for each core. Cores were placed in sterile plastic bags, transported back to the lab on ice and stored at 4 °C.

Seagrass plants were removed from surrounding sediment using an 850 µm (size 20) stainless steel sieve and separated by species within 24 h of collection. Fresh shoot galls were mounted in distilled water, crushed, and examined using a Nikon Eclipse 80 microscope equipped with Nomarski interference contrast optics. Digital photographs of microscopic structures were taken using a SPOT Insight camera and measurements were made using SPOT 4.1 software. Infected and healthy specimens were preserved in 50% alcohol. A dried voucher specimen and a microscope slide voucher were deposited in the herbarium of the University of Southern Mississippi's Gulf Coast Research Laboratory (HGCRL) (Ocean Springs, MS).

Results and Discussion

No infected *H. wrightii* plants were collected at Pointe aux Pines, AL (site 1). One core out of 6 collected at Grand Bay NERR, MS (site 2) and one core out of 6 collected at Chandeleur Islands, LA (site 3) contained *H. wrightii* infected with *P. diplantherae*. In the infected cores, 53% of 103 *H. wrightii* shoots collected at the Grand Bay NERR exhibited infection, and 100% of 3 shoots collected at the Chandeleur Islands were infected. Additional *H. wrightii* specimens exhibiting *P. diplantherae* infection were collected from Horn Island, MS (site 4) in October 2006 and from Chandeleur Islands, LA in June 2008 (64% of 22 shoots infected).

Infected specimens of *H. wrightii* closely resembled the descriptions and illustrations of Ferdinandsen and Winge (1914) and illustrations redrawn by Karling (1968). Host shoot tissue was transformed into white galls at the internodes in infected specimens, and host plant rhizomes and shoots were stunted and disfigured when compared with uninfected specimens (Figure 2). Gall diameter ranged from 1-3 mm. In actively growing shoots, galls were white to light cream near the shoot apex as in Braselton and Short (1985). However, the current study observed reddish brown galls further away from the apex (Figure 3), whereas Braselton and Short (1985) noted these galls were brown. Host gall tissue was brittle, breaking easily to release abundant smooth-walled, red-brown globose spores measuring 4-4.6 µm in diameter (Figure 4). Biflagellate zoospores were not observed. Not all shoots on a given rhizome were infected with *P. diplantherae*. One plant exhibited 4 healthy shoots next to 4 infected shoots on one rhizome.

Similar water temperatures, pH readings and supersaturated dissolved oxygen concentrations were recorded at sites 1-3. Water depth was shallower at site 1 (0.60 m) than at site 2 (0.90 m) and site 3 (1.05 m). *Plasmodiophora diplantherae* was not observed at site 1, which suggests that the parasite may require deeper water.

Den Hartog (1989) noted that species of the seagrass genus *Zostera* similarly infected with the related parasite *P. bicaudata* have noticeably stunted roots, allowing the plants to become uprooted easily. During collecting trips for the

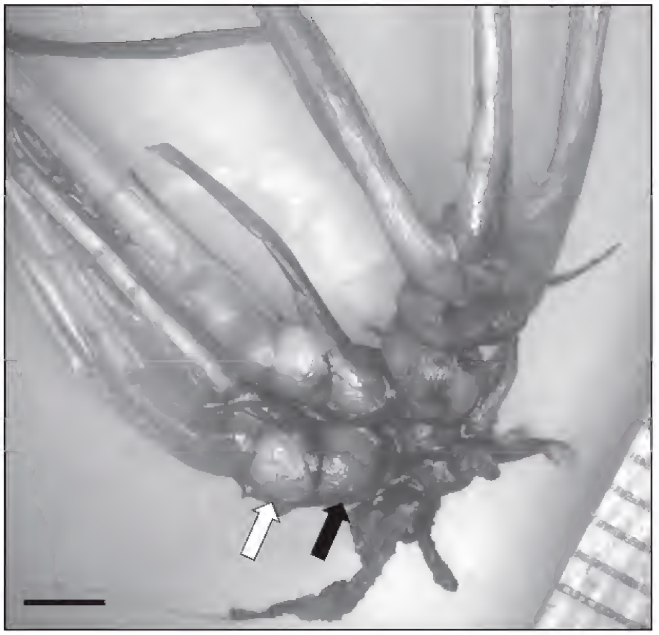

Figure 3. *Swollen internodes (galls) of* Halodule wrightii *infected with* Plasmodiophora diplantherae. *Galls closer to shoot apices were white-cream (white arrow), while galls closer to rhizome were reddish-brown (black arrow). Scale bar = 2 mm.*

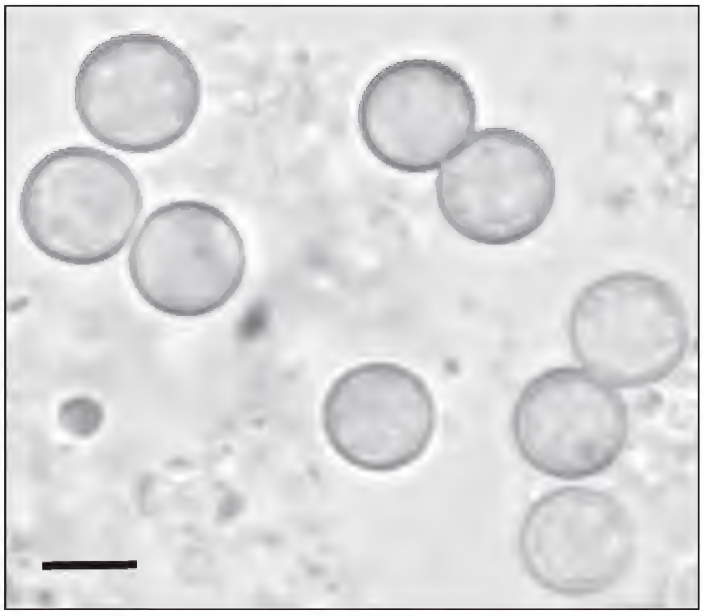

Figure 4. *Resting spores of* Plasmodiophora diplantherae. *Scale bar = 4 µm.*

current study, numerous floating *H. wrightii* plants were observed at all sites where this parasite was collected. Seagrass uprooting could be a serious problem facing these declining habitats, and uprooting may also aid in the dispersal of *P. diplantherae*. Additionally, seagrass uprooting may be partially to blame for the low establishment rate (<50%; Fonseca et al. 1998) of current seagrass restoration projects. Seagrass restoration scientists in the northcentral GOM should be aware of the presence of this parasite. Factors determining the pattern of occurrence of *P. diplantherae* are currently unknown.

This study detected *P. diplantherae* in both fall and spring in the northcentral GOM over the course of three years. *Plasmodiophora bicaudata* is thought to overwinter in the rhizomes of *Zostera* plants (den Hartog 1989) and whether *P. diplantherae* shares this ability is unknown. However, plasmodiophorids can persist in the environment as resting spores, which were detected in this study within *H. wrightii* galls. Chlorine has demonstrated success in inactivating plasmodiophorid resting spores (Datnoff et al. 1987), and may hold promise for treating infected *H. wrightii* raised for future restoration activities.

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Macrofauna Associate with Ungrounded Prop Roots of *Rhizophora mangle* in Veracruz and Quintana Roo, Mexico

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SHORT COMMUNICATION

MACROFAUNA ASSOCIATED WITH UNGROUNDED PROP ROOTS OF *RHIZOPHORA MANGLE* IN VERACRUZ AND QUINTANA ROO, MEXICO

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Introduction

The prop roots of the red mangrove (*Rhizophora mangle*) provide a solid substrate for diverse assemblages of marine organisms in areas typically characterized by soft bottoms (Bingham 1992, Farnsworth and Ellison 1996). Macrobenthic communities of mangroves have received little attention compared with other components of the ecosystem, largely due to sampling difficulties (Lee 2008). Mangrove root epifauna are likely used by predatory fish, especially juveniles. Thus, these organisms have the potential of being important links between mangroves and adjacent ecosystems. The fauna associated with red mangrove prop roots along Mexican Gulf of Mexico (GOM) and Caribbean shorelines has not been well described. The infauna of red mangrove associated sediments has been studied in GOM sites in the Río Carrizal Estuary, Tamaulipas, Mexico (Rabalais et al. 1989), Laguna de Términos, Veracruz, Mexico (Hernández-Alcántara and V. Solís-Weiss 1995) and Rookery Bay, Florida (Sheridan 1997). Red mangrove root epifauna in the GOM has been described only in Laguna de Tamiahua (Fajardo M. 1990). Although red mangrove root faunas have been described in some areas of the Caribbean, such as Puerto Rico (Mattox 1949, Kolehmainen and Wildner 1975) and Bahia de Buche, Venezuela (Sutherland 1980), in the northwestern Caribbean the mangrove root epifauna has only been described in Belize (Ellison and Farnsworth 1992, Farnsworth and Ellison 1996). The objective of this study was to describe macrofaunal community composition of ungrounded red mangrove prop roots in the southwestern GOM and the northwestern Caribbean, on the Yucatan Peninsula. The communities we describe are compared to others in Mexico, Central America and the wider Caribbean to address factors that may explain similarities and differences.

Study Area

Laguna La Mancha is located on the central GOM coast of Mexico about 51 km north of the city of Veracruz, in the state of Veracruz, Mexico (Figure 1). The lagoon is located behind a barrier peninsula with only one small outlet to the GOM that is closed during the dry season, October to May (Moreno-Casasola et al. in press). Freshwater enters the lagoon via the Río Caño Grande to the south and a small, ephemeral stream to the north; fresh groundwater is also a major water source for the lagoon. In Laguna La Mancha, salinity ranged from 18 ppt (Site 4) to 25 ppt (Site 3) during collections. Water depth ranges from 0.5 - 1.0 m (Contreras 1993).

The Caribbean study area was located within the Sian Ka'an Biosphere Reserve on the Yucatan Peninsula in the state of Quintana Roo, Mexico. Mangrove roots were collected from sites within a lagoon system behind a long (56 km) barrier peninsula (Figure 1). Laguna Campechén, Laguna Boca Paila, and Laguna San Miguel comprise the northern portion of the lagoon system. These lagoons are only connected to the more marine-influenced southern portion by a small mangrove channel and to the Caribbean through a single narrow opening (~100 m) at Boca Paila (Sanvicente-Añorve et al. 2002). The entire lagoon system is supplied with freshwater from subterranean sources and both Laguna Campechén and Laguna San Miguel are isolated from marine influence. Salinity in Laguna Boca Paila ranged from 19 ppt (Site 3) to 33 ppt (Site 5) during collections. Water depth within the lagoons is between 0.5 - 1.0 m except in channels near the inlet (Boca Paila) where tidal scouring has occurred (Tunnell et al. 1993). For the purposes of this paper, the northern portion of the lagoon system will be referred to collectively as Laguna Boca Paila.

Materials and Methods

Ungrounded prop roots of red mangroves were randomly collected from 5 sites in both Laguna La Mancha and Laguna Boca Paila that were selected because they varied in distance from the inlet. Collections were made at both sites during the transition from the wet to dry season: May 8-11, 1999 at Laguna Boca Paila and May 26, 1999 at Laguna La Mancha. Roots were collected from water depths < 1 m, ranging from 16-29 cm in Laguna La Mancha and 42-94 cm in Laguna Boca Paila. Roots were randomly selected by reaching into the root mass and grabbing a root. If it was ungrounded, it was collected. Working in one di-

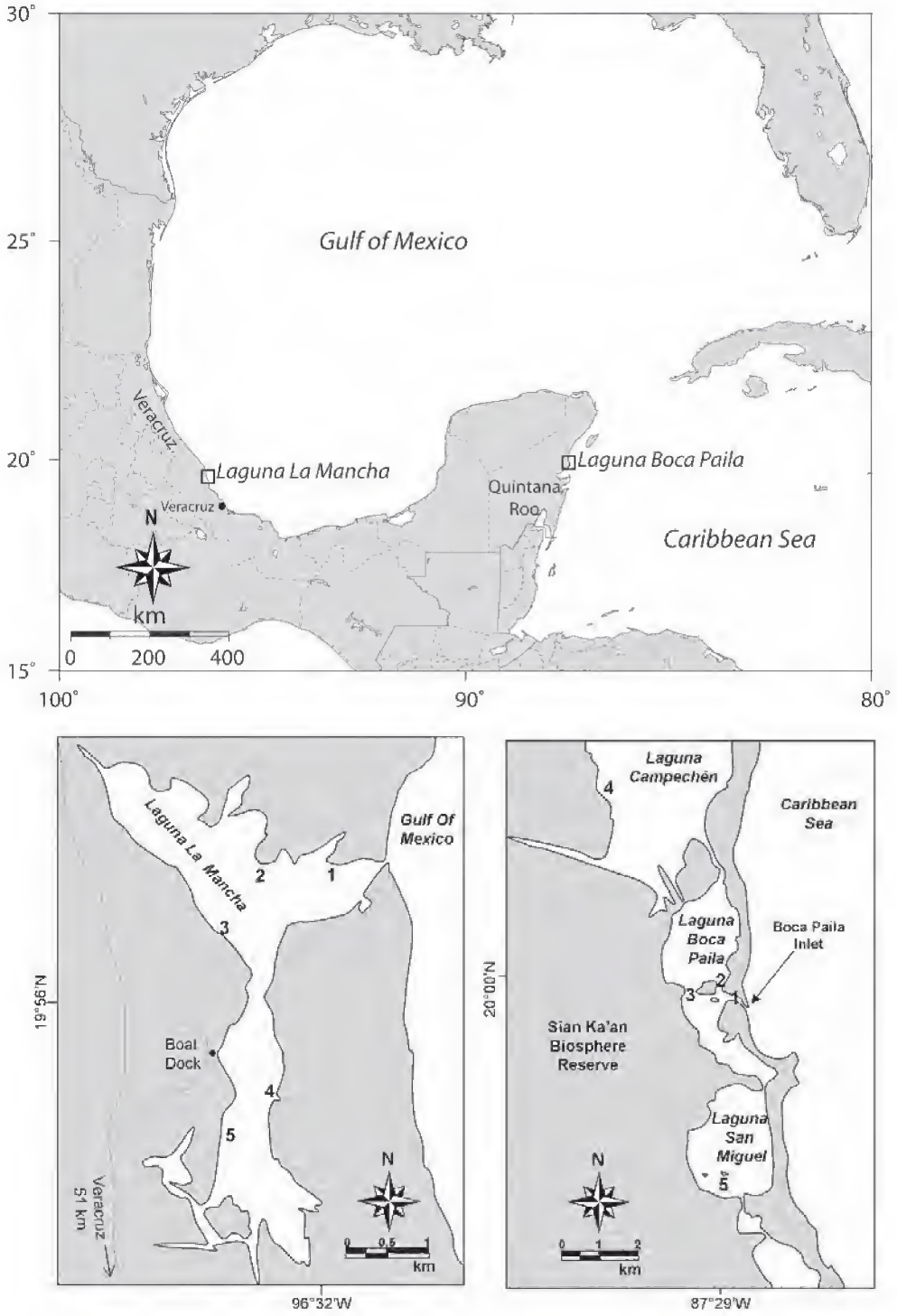

Figure 1. *Map showing the general locations of mangrove study areas within the Mexican Gulf of Mexico and Caribbean Sea. The approximate locations of sites where red mangrove roots were collected are shown within Laguna La Mancha, Veracruz (bottom left) and the northern lagoon system in the Sian Ka'an Biosphere Reserve, Quintana Roo (bottom right).*

rection along the edges of the mangroves at a site, 10 steps were taken, and another root was collected in the same way. This sequence was followed until 10 roots were collected at each site (50 roots total for each study area). Roots were collected by cutting with lopping shears just above the high tide line demarcated by the upper limit of dead barnacles. Before cutting the roots, a 0.5 mm mesh biobag was placed around the root to prevent fauna from escaping. All samples were fixed in 10% buffered formalin for 2 d then transferred to 45% isopropyl alcohol for storage until analysis.

In the laboratory, algae and fauna were removed from the roots, and fauna were separated from the algae. Both were stored in 45% isopropyl alcohol. Total algal biomass was determined for each root by drying algae at 100 °C in a preweighed pan for 72 h. Faunal organisms from each root were identified to the lowest practical taxon and counted. A lack of regional taxonomic sources meant that some polychaetes could only be identified to family, and some amphipod and polychaete genera could not be identified to species; these were designated as "A", "B", etc., when it was clear that there was more than one unidentifiable, but different species in the same genus. Other taxa, such as insects, were identified only to order, except for the dipteran family Dolichopodidae. In other cases, specimens were very small, appeared to be juveniles, or were missing parts preventing their identification to lower than family or genus. In most cases, taxa that were not identified to species represented relatively rare organisms that were present on only one or a few roots. Published references used to identify organisms included: polychaetes – Uebelacker and Johnson (1984); crabs – Felder (1973), Williams (1984), Chaney (1999); amphipods – Bousfield (1973), Barnard and Karaman (1991); isopods – Kensley and Schotte (1989). References used to determine phylogenetic order of organisms included polychaetes – Rouse and Pleijel (2001); gastropods – Rosenberg (2007); bivalves – Mikkelson and Bieler (2008); crustaceans – Martin and Davis (2001).

Root length (cm) was measured with a metric ruler. Diameter (cm) along the top and bottom of each root was determined with vernier calipers and mean diameter was

calculated. Surface area (cm^2) of the root was estimated by using the length and mean diameter of each root. This was accomplished by using the formula for obtaining the surface area of a cylinder (π x diameter x length; Sheridan 1992). Estimated root surface area averaged 230.8 ± 113.6 cm^2 (mean ± sd) at Laguna La Mancha and 221.9 ± 143.9 cm^2 at Laguna Boca Paila with an overall mean of 226.3 ± 128.5 cm^2. Based on these estimates, we standardized invertebrate counts and algal biomasses to numbers or g dry weight per 100 cm^2.

Results

Forty-seven invertebrate taxa and 8,811 individuals were collected from roots in Laguna La Mancha (Table 1). The bivalves *Mytilopsis leucophaeta*, *Crassostrea virginica*, and *Ischadium recurvum* were the dominant species overall and constituted 98% of molluscs and 49% of all invertebrates collected. Roots were divided into those that were dominated by *C. virginica* (≥20/100 cm^2) and those that were not. Molluscs and amphipods were the most abundant groups regardless of root type. The amphipod assemblage was dominated by *Hyale prevostii*, *Melita nitida*, and *Amphilochus menehune*. *Cassidinidea ovalis* was the most abundant isopod. Polychaetes were not abundant. *Polysiphonia* sp. was the only algal genus present and algae were found on only seven roots. Roots dominated by *C. virginica* were more diverse than those that were not (44 taxa vs 35). In addition, many taxa, particularly polychaetes, bivalves, and amphipods, were more abundant on roots dominated by oysters. These roots were often colonized by oysters throughout their length, with no noticeable zonation. On roots with fewer oysters, the only apparent zonation was the concentration of barnacles at the top of the root in the intertidal zone.

In Laguna Boca Paila, 56,536 invertebrates were collected and 57 taxa were identified (Table 2). Most roots (88%) were colonized by algae and five species were identified: *Acetabularia crenulata*, *Batophora oersteddi*, *Anotrichium tenue*, *Bostrychia montagnei*, *Polysiphonia* sp. Mean algal biomass at Site 1 was 0.1494 g/100 cm^2, less than half the algal biomass recorded at the next lowest site (Site 5, 0.3181 g/100 cm^2) and only 12% of the highest mean algal biomass recorded (Site 3, 1.2594 g/100 cm^2). Algal biomass of individual roots with algae ranged from 0.0005 g/100 cm^2 to 2.2052 g/100 cm^2. Roots were divided into those that were dominated by algae (≥1 g dry weight/100 cm^2) and those that were not. Amphipods and isopods dominated both root types, although several species of amphipods, notably *Hyale plumulosa* and *Erichthonius brasiliensis*, were much more abundant on roots dominated by algae than on roots with little or no algal growth. Densities of *Sphaeroma terebrans* were similar on both root types. *Ischadium recurvum* and *M. leucophaeata* were the dominant molluscs and both were substantially more abundant on algae-dominated roots. The polychaete assemblage was comprised of 13 species but only *Capitellides*

TABLE 1. *Density (#/100 cm^2, with se in parenthesis) of taxa associated with ungrounded red mangrove roots collected from Laguna La Mancha, Veracruz on the Mexican Gulf Coast.*

	Oyster-dominated (n = 35)	Bare (n = 15)
Nemertea	0.5 (0.2)	0.1 (0.1)
Polychaetes		
Capitella capitata	1.8 (0.8)	1.2 (0.4)
Maldanidae Species A	0.3 (0.1)	0.2 (0.1)
Laeoneris culveri	9.3 (7.0)	0.4 (0.3)
Nereis falsa	20.5 (9.4)	3.9 (1.0)
Ehlersia sp.	0.1 (0.1)	0.1 (0.1)
Alciopidae Species A		0.2 (0.1)
Dorvilleidae Species A	0.2 (0.2)	0.1 (0.1)
Eunicidae Species A	0.1 (0.1)	
Marphysa sanguinea	2.5 (2.3)	
Serpula sp.	0.6 (0.2)	0.5 (0.4)
Boccardia hamata	2.7 (2.2)	0.6 (0.3)
Streblospio benedicti	0.2 (0.1)	
Gastropods		
Neritina virginea	1.0 (0.7)	0.3 (0.2)
Boonea impressa	3.1 (2.3)	
Bivalves		
Geukensia demissa	0.1 (0.1)	0.1 (0.1)
Ischadium recurvum	65.8 (15.4)	15.2 (4.6)
Isognomon bicolor	0.1 (0.1)	
Crassostrea virginica	83.4 (15.4)	14.1 (3.7)
Lucinidae Species A	0.1 (0.1)	
Mytilopsis leucophaeata	111.0 (22.5)	50.5 (24.1)
Bankia sp.	2.7 (0.8)	1.6 (1.1)
Barnacles		
Balanus spp.	47.2 (10.9)	23.7 (6.7)
Amphipods		
Amphilochus menehune	18.2 (4.7)	17.7 (7.4)
Amphithoe Species A	7.9 (3.8)	0.4 (0.4)
Corophium volutator	17.8 (4.8)	9.4 (3.5)
Gammarus mucronatus	3.2 (2.9)	1.2 (1.1)
Gammarus Species A	0.1 (0.1)	0.1 (0.1)
Ericthonius brasiliensis	1.0 (0.5)	1.7 (1.7)
Hyale plumulosa	0.6 (0.6)	
Hyale prevostii	33.1 (13.9)	38.6 (31.6)
Parhyale fascigera	0.1 (0.1)	
Elasmopus sp.	1.3 (0.9)	
Melita nitida	24.9 (6.1)	4.7 (1.4)
Orchestia gammarella	0.1 (0.1)	
Isopods		
Aega sp.		0.1 (0.1)
Cassidinidea ovalis	31.8 (6.4)	12.3 (3.5)
Uromunna caribea	0.7 (0.2)	1.1 (0.8)
Tanaids		
Hargeria rapax	12.5 (8.6)	11.5 (6.5)
Crabs and Shrimp		
Macrobrachium sp.	0.4 (0.2)	2.3 (1.3)
Brachyuran larvae	0.5 (0.3)	1.5 (1.5)
Micropanope nuttingi	0.1 (0.1)	
Panopeus herbstii	0.4 (0.2)	
Pachygrapsus gracilis	13.2 (2.4)	4.6 (1.1)
Armases ricordi		0.1 (0.1)
Insects		
Coleoptera	0.3 (0.2)	0.1 (0.1)
Diptera		
Dolichopodidae	0.3 (0.2)	0.4 (0.3)

TABLE 2. *Density (#/100 cm², with se in parenthesis) of taxa associated with ungrounded red mangrove roots collected from Laguna Boca Paila, Quintana Roo, on the Mexican Caribbean coast.*

	Algae-dominated (n = 23)	Not algae-dominated (n = 27)
Cnidarians		
Actiniara (anemone)		0.5 (0.4)
Nemertea	0.4 (0.2)	0.1 (0.1)
Polychaetes		
Capitellides jonesi	11.8 (4.5)	3.2 (1.9)
Ceratonereis mirabilis	0.3 (0.3)	1.9 (1.9)
Ceratonereis singularis		0.5 (0.3)
Neanthes acuminata		0.8 (0.6)
Nereis pelagica	0.4 (0.4)	0.5 (0.3)
Platynereis dumerilii	0.7 (0.4)	1.2 (0.7)
Syllidae Species A		0.2 (0.2)
Lysidice ninetta	0.1 (0.1)	
Marphysa sanguinea	1.3 (0.7)	0.1 (0.1)
cf. *Neovermilia capensis*	17.4 (9.4)	8.9 (4.8)
Aonides paucibranchiata	0.1 (0.1)	0.1 (0.1)
Minuspio cirrobranchiata	1.3 (1.1)	0.7 (0.5)
Gastropods		
Cerithiidae Species A	0.6 (0.6)	0.9 (0.6)
Echinolittorina lineolata	0.1 (0.1)	0.6 (0.3)
Littoraria angulifera	0.1 (0.1)	
Janthina sp.		0.1 (0.1)
Bullidae Species A	0.2 (0.2)	
Bivalves		
Ischadium recurvum	106.4 (70.2)	48.1 (28.6)
Isognomon alatus	0.1 (0.1)	0.4 (0.2)
Isognomon bicolor	1.3 (1.3)	
Mytilopsis leucophaeata	23.9 (10.8)	12.9 (7.9)
Teredo sp.	0.6 (0.4)	7.1 (2.9)
Barnacles		
Balanus spp.	10.0 (5.8)	11.9 (6.6)
Amphipods		
Amphilochus menehune	149.2 (91.8)	94.5 (47.2)
Amphithoe Species B	109.1 (79.4)	2.6 (2.3)
Amphithoe Species C		0.2 (0.2)
Cymadusa filosa	0.1 (0.1)	3.9 (3.3)
Corophium Species A	5.4 (5.1)	27.8 (12.9)
Corophium Species B	3.9 (3.5)	25.9 (11.3)
Ericthonius brasiliensis	548.1 (231.4)	169.9 (117.0)
Hyale plumulosa	1569.1 (541.2)	276.0 (147.8)
Parhyale fascigera	100.0 (45.5)	107.5 (43.6)
Lysianassa alba	37.8 (16.4)	40.1 (28.1)
Maera inaequipes	1.6 (0.9)	14.7 (7.3)
Melita nitida	34.8 (15.9)	8.2 (3.3)
Isopods		
Cyathura cubana	9.2 (5.3)	13.0 (7.2)
Excorallana tricornis tricornis	6.7 (2.4)	10.0 (4.3)
Cassidinidea ovalis	2.2 (1.2)	17.5 (6.6)
Sphaeroma terebrans	103.4 (40.4)	96.2 (26.0)
Dynamenella perforata	0.3 (0.2)	1.4 (1.1)
Munnidae Species A	6.4 (1.7)	21.3 (8.4)
Uromunna caribea	32.0 (12.1)	12.4 (4.3)
Tanaids		
Tanais sp.	8.6 (3.5)	24.3 (8.3)
Hargeria rapax	97.4 (30.5)	32.6 (13.9)
Crabs and Shrimp		
Palaemonetes sp.	1.3 (0.6)	0.8 (0.5)
Paguridae Species A		0.1 (0.1)
Brachyuran larvae	2.8 (1.0)	0.4 (0.2)
Majiidae Species A		0.2 (0.2)
Panopeus herbstii	11.1 (3.9)	2.0 (1.6)
Grapsidae Species A		0.1 (0.1)
Pachygrapsus gracilis	2.5 (1.1)	0.7 (0.5)
Sesarma curacaoense		0.2 (0.1)
Insects		
Hemiptera	0.1 (0.1)	0.1 (0.1)
Diptera		0.1 (0.1)
Hymenoptera		0.1 (0.1)

jonesi and cf. *Neovermilia capensis* were common. Roots dominated by algae were less diverse than those that were not (45 taxa vs 54). Roots dominated by algae were typically covered from top to bottom, often with very luxuriant growth. Like Laguna La Mancha, the only noticeable zonation pattern was the concentration of barnacles at the top few centimeters of the root where it entered the water.

Discussion

The main differences between the Laguna La Mancha and Laguna Boca Paila were the dominance of bivalves, particularly oysters, on root faunas in Laguna La Mancha and the widespread colonization of roots by algae in Laguna Boca Paila, with dominance by crustaceans, especially amphipods and the root boring isopod *S. terebrans*. Neither site exhibited noticeable faunal zonation, probably because waters were shallow and tidal fluctuation low. The sponges, tunicates and hydroids that are characteristic of many mangrove root faunas (e.g., Ellison and Farnsworth 1992, Sutherland 1980) were conspicuously absent from both sites. In addition, the faunas we describe include many more polychaetes and amphipods. Tube-dwelling sabellids and serpulids were the only polychaetes reported from roots in Belize (Ellison and Farnsworth 1992). Mattox (1949) noted the presence of the spionid *Polydora* in addition to a sabellid (*Sabella*) and a serpulid (*Hydroides*). Neither Mattox (1949) or Ellison and Farnsworth (1992) reported any amphipods.

Oyster-dominated roots were found only in Laguna La Mancha. Oyster coverage increased available surface area and provided more attachment sites for sessile organisms such as mussels and serpulid polychaetes as well as refuge for motile species (e.g., *Pachygrapsus gracilis, Nereis falsa*). The habitat complexity added by the oysters increased species richness and organisms were generally more abundant. For example, although the difference in polychaete species richness between root types was minimal (11 vs 9), the three most abundant species on oyster-dominated roots were 4.5 to 23 times more abundant than on bare roots. Abundance of some amphipod species was similar between bare and oyster-dominated roots (e.g., *Amphilochus menehune*, *H. prevostii*). However, most amphipod species were substantially more abundant on oyster-dominated roots and species richness was greater (12 vs 8). The fauna from Laguna La Mancha was very similar to that found in Laguna Tamiahua (Fajardo M. 1990), about 200 km to the north. Both lagoons are sites of artisanal oyster culture, which contributes greatly to oyster dominance of root faunas.

Algae-dominated roots were found only in Laguna Boca Paila. Where salinity, turbidity and water temperature were low (especially at Site 3 near the cenote) algal growth flourished, providing habitat for a plethora of small, motile invertebrates, mainly amphipods. Species richness was lower on algae-dominated roots than on roots with less algae but

many species that were present on both types of roots were more abundant on algae-dominated roots. This was particularly true of the polychaetes, most bivalves, and about half of the amphipod species. All species of isopods identified were found on both root types. Abundance of the root-boring isopod *S. terebrans* was similar on both root types so algae did not appear to inhibit their colonization. However, with the exception of *Uromunna caribea*, the rest of the non-boring isopod species were more abundant on roots that were not dominated by algae.

Mangroves adjacent to offshore coral reefs are supplied with invertebrate larvae produced by reef inhabitants. Rützler (1969) noted that the sessile fauna of mangroves at Low Isles near the Great Barrier Reef "clearly belonged to the reef fauna proper." Root faunas on red mangrove prop roots in many lagoons isolated from coral reefs lack this diverse invertebrate larval supply and are often dominated by boring isopods, oysters and mussels (Perry 1988, Fajardo M. 1990). In Belize, epibiont species richness increased as proximity to the barrier reef increased, especially with regard to algae, sponges, hydroids, and ascidians; cover of these groups was also greater at sites nearer the reef (Ellison and Farnsworth 1992). In the Gulf of Nicoya, Costa Rica, the root-burrowing isopod *Sphaeroma peruvianum* and encrusting barnacle *Balanus* spp. were the dominant faunal components whereas sponge and tunicate coverage of roots was very low (Perry 1988). Bivalves, sponges, and tunicates were common faunal components in Puerto Rican mangrove lagoons with little oceanic communication (Mattox 1949) and in Bahía de Buche, a protected bay fringed by mangroves on the northern Venezuelan coast (Sutherland 1980).

The lack of sponges, tunicates and hydroids on mangrove roots in Laguna La Mancha and Laguna Boca Paila was likely due to a combination of isolation from larval sources (especially in the GOM) and salinities that are generally lower than marine. Salinity is an important determinant of mangrove faunal composition (Walsh 1967, Ellison and Farnsworth 1992). In all studies of mangrove root fauna in which sponges, hydroids and/or tunicates were prominent, salinities were marine (Mattox 1949, Sutherland 1980, Perry 1988, Bingham 1992, Ellison and Farnsworth 1992). Salinities in Puerto Rican mangrove lagoons typically mirrored those of the adjacent Atlantic Ocean (Mattox 1949). In Placencia Lagoon, Belize, where salinities were < 30 ppt and variable, only two sponge species were identified, the red alga *Bostrychia* was abundant, and no ascidians or cnidarians were reported (Ellison and Farnsworth 1992). Survival of species of sponges and tunicates common on mangrove roots near reefs was low when they were transplanted into Placencia Lagoon.

Salinities measured during this study were generally within published ranges for both sites. Laguna La Mancha is an estuarine coastal lagoon with salinities averaging 19.7 ppt and ranging from 11.5-25 ppt (Contreras-Espinoza and Warner 2004). This lagoon is also very turbid with low chlorophyll *a* values and net productivity relative to most other estuaries on the Mexican Gulf Coast. Laguna La Mancha and Laguna Tamiahua share similar hydrologic characteristics as well as similar mangrove root faunas. Salinities in the northern portion of the lagoon system in the Sian Ka'an Biosphere Reserve averaged 4.5-17.8 ppt during October and February, and 4.5-24.8 ppt during May and August (Sanvicente-Añorve et al. 2002). The ichthyoplankton assemblage in the northern half of the lagoon system (Campechén, Boca Paila, San Miguel) was dominated by estuarine components, unlike the assemblage in the southern portion lagoon system which was described as oceanic. The mangrove root fauna in the northern portion of the lagoon system could similarly be described as estuarine. The macrofaunal communities of ungrounded mangrove prop roots in Laguna La Mancha and Laguna Boca Paila described in this study reflect the estuarine conditions of the lagoons from which they were collected. Although both lagoons are isolated to varying degrees from potential sources of reef associated fauna, salinities that average less than marine are likely a major force determining community composition.

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Age Estimates of Two Large Misty Grouper, *Epinephelus mystacinus* (Serranidae) from Bermuda with a Comparison of the Age of Tropical Groupers in the Western Atlantic

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SHORT COMMUNICATION

AGE ESTIMATES OF TWO LARGE MISTY GROUPER, *EPINEPHELUS MYSTACINUS* (SERRANIDAE) FROM BERMUDA WITH A COMPARISON OF THE AGE OF TROPICAL GROUPERS IN THE WESTERN ATLANTIC

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Introduction

The Bermuda Seamount is the most northerly known location for the misty grouper (*Epinephelus mystacinus*) in the western North Atlantic, but this species is broadly distributed as far south as Trinidad, as well as in the Galapagos Islands in the eastern Pacific (Heemstra and Randall 1993). Adults are reported to be solitary, deep-water predators in the depth range 100-400 m, and are thought to prefer high relief, hard bottom slope habitats (Bullock and Smith 1991). They are presumed to be protogynous hermaphrodites and slow-growing, characteristics that are common in the epinepheline groupers (Heemstra and Randall 1993). In Cuba, they are an incidental catch in the deep-water fishery that primarily targets snappers (Lutjanidae) (Claro et al. 2001) and have been reported to be the dominant grouper in the deep-water fishery in the US Virgin Islands (Heemstra and Randall1993). An active fishery has now developed in Puerto Rico (E. Pineiro, pers. comm., Caribbean Fishery Management Council, Puerto Rico); landings of misty grouper from 2004-2006 ranged from 2,175–3,361 kg (G. Garcia-Moliner, pers. comm., Caribbean Fishery Management Council, Puerto Rico). Misty grouper are included in Grouper Unit 4 in the Caribbean Fishery Management Council's Management Plan. The six species in this unit are considered overfished but misty grouper are excluded from current management action (G. Garcia-Moliner, pers. comm.). However, considering the developing fishery in Puerto Rico, it is apparent that more information is needed about the biology of this species to help guide management decisions.

Groupers were the largest category in the fishery landings in Bermuda until the mid-1970s when stocks of many grouper species became severely overfished (Luckhurst 1996; Smith-Vaniz et al. 1999). As a result, local fishermen commenced fishing in deeper water (270–360 m) in an attempt to maintain grouper catch levels; the most common species taken was the misty grouper (peaked at 7,400 kg in 1979; Luckhurst 1996). By 1981, misty grouper landings declined substantially and fishermen started setting lines in greater depths (to 650 m) to target wreckfish, *Polyprion americanus* (B. Luckhurst, pers. obs). These two species plus deep-water lutjanids were subjected to intense fishing pressure around the Bermuda platform, and suffered precipitous declines when the limited habitats (depth strata) for these species were overexploited (Luckhurst 1996).

Misty grouper are known to attain at least 54 kg and 115 cm total length (TL) (Heemstra and Randall 1993) although a maximum size of 160 cm TL is reported (Appeldoorn et al. 1987, cited in www.FishBase.com). Recently, two specimens (152 and 157 mm TL) were landed by commercial fisherman from the edge of the Bermuda platform, providing documentation of the maximum size which may be attained by this species. There are no age and growth studies on misty grouper (Heemstra and Randall 1993; www.FishBase.com). Therefore, the age data presented here, although not validated, represent the first estimates of maximum longevity and support Campana's (2005) statement that, "methods for validating ages of deep-sea fishes are urgently required." We compared the age estimates of our misty grouper specimens with the age of a large wreckfish (45.5 kg) taken from a similar but deeper-water habitat. In addition, we provide a comparison of the maximum ages of 9 other species of grouper in the western Atlantic.

Materials and Methods

Both misty grouper specimens were caught on commercial vertical longline gear set in 220-300 m around the edge of the Bermuda platform in 2000 and 2001, whereas the wreckfish was caught in about 650 m using the same gear type in 1995. The TL (cm) and whole weight (kg) were recorded for each fish and the sagittal otoliths were removed, washed, dried and weighed. Transverse sections were cut, polished and decalcified with saturated EDTA at a pH of 7.4 (Secor et al. 1991). We initially made counts of the opaque zones (interpreted as annuli) on the transverse sections at 40X magnification with a light microscope to establish initial age estimates. As a result of the very closely spaced and large number of increments visible, we then increased to

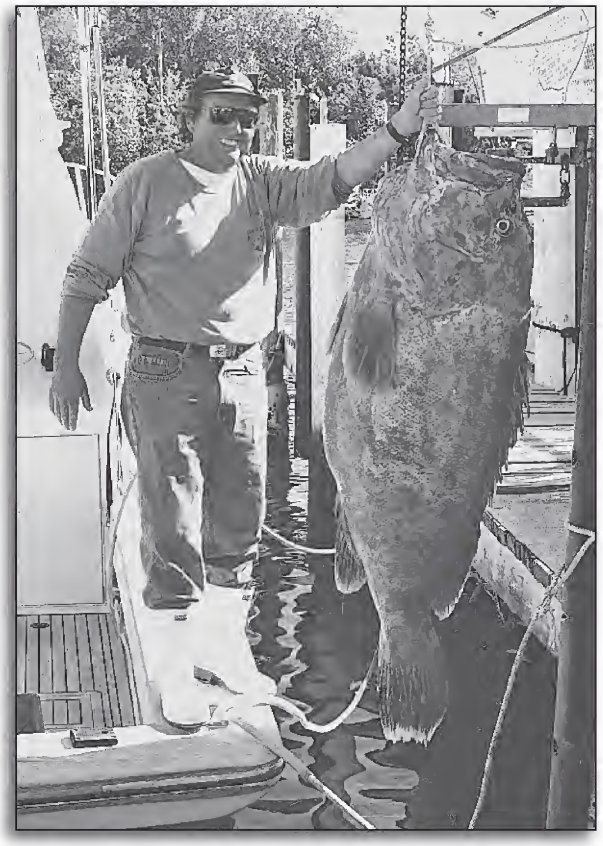

Figure 1. *Photograph of 75.5 kg misty grouper caught by Alan DeSilva in March 2000. Photo by Craig Soares.*

100X magnification in order to achieve better resolution. We made repeated independent counts of the increments observed at 100X and also did counts on a Scanning Electron Microscope (SEM) at 160X. There was good correspondence between our counts at 100X (98%) and 160X (97%). Our final age estimates were agreed through discussion and consensus. Although radiometric dating is one of the best methods for age estimation in long-lived fish (Morales-Nin and Panfili 2005), this technique was not available to us at the time this research was conducted.

The otoliths of many deep-water species show growth zones that are similar to those interpreted as annuli in shallow water species (Bergstad 1995, Morales-Nin and Panfili 2005). We interpreted the increments that we observed in these sagittal preparations in the same manner that we have employed in studies of shallow water species in Bermuda, e.g. lane snapper, *Lutjanus synagris* (Luckhurst et al. 2000) and black grouper, *Mycteroperca bonaci* (B. Luckhurst, unpublished data).

Finally, as we were not able to validate the increments as annuli, we compared our misty grouper otolith microstructure to that of the wreckfish (a similar deep-water species) prepared using the identical protocol. The micro-morphology of the otoliths of the two species is very similar and the results we obtained are consistent with the findings of Peres and Haimovici (2004) in their study of southwestern Atlantic wreckfish.

Results and Discussion

The two misty groupers were caught in two consecutive years (Table 1), but in different sections off the edge of the Bermuda platform, whereas the wreckfish was caught five years earlier. It is interesting to note the similarity in the length and weight of the two misty grouper (Table 1), both caught in similar depths. One of the striking aspects of the misty grouper is its large girth (Figure 1), which contributes significantly to its weight. The capture of these two misty grouper specimens has increased the documented maximum weight for the species by over 20 kg (Heemstra and Randall 1993). The TL of the larger specimen is only 3 cm shorter than the maximum reported for the species (Appeldoorn et al. 1987).

The estimated ages of the misty groupers were: specimen #1 (157 cm) - 150 years and specimen #2 (152 cm) - 135 yrs (Table 2). As there are no age estimates available in the literature for misty grouper (Heemstra and Randall 1993) with which to compare the ages of our two specimens, we are only able to evaluate our estimates in comparison with other serranid species that have been aged (Table 2). In the absence of validation, the Bermuda wreckfish was included to provide a basis of comparison for a similar deep-water species from the same general location. The otolith microstructure of the misty grouper and wreckfish (Figure 2) were very similar. As a result, we interpreted the increments (opaque zones) that we counted in both the misty grouper and the wreckfish as annuli. We estimated the age of our wreckfish at 60 yrs; since the oldest published age of a wreckfish is 81 yrs (Peres and Haimovici 2004), our estimate seemed reasonable.

A number of studies (Dwyer et al. 2003, Kerr et al. 2004,

TABLE 1. *Capture details and sizes of two misty groupers and one wreckfish from Bermuda whose ages were estimated using sectioned sagittal otoliths.*

Species	Date of capture	Depth of capture (m)	Whole weight (kg)	Total length (cm)
Misty grouper #1	March 11, 2000	270	75.5	157
Misty grouper #2	April 12, 2001	220	74.5	152
Wreckfish	October 1, 1995	650	45.5	134

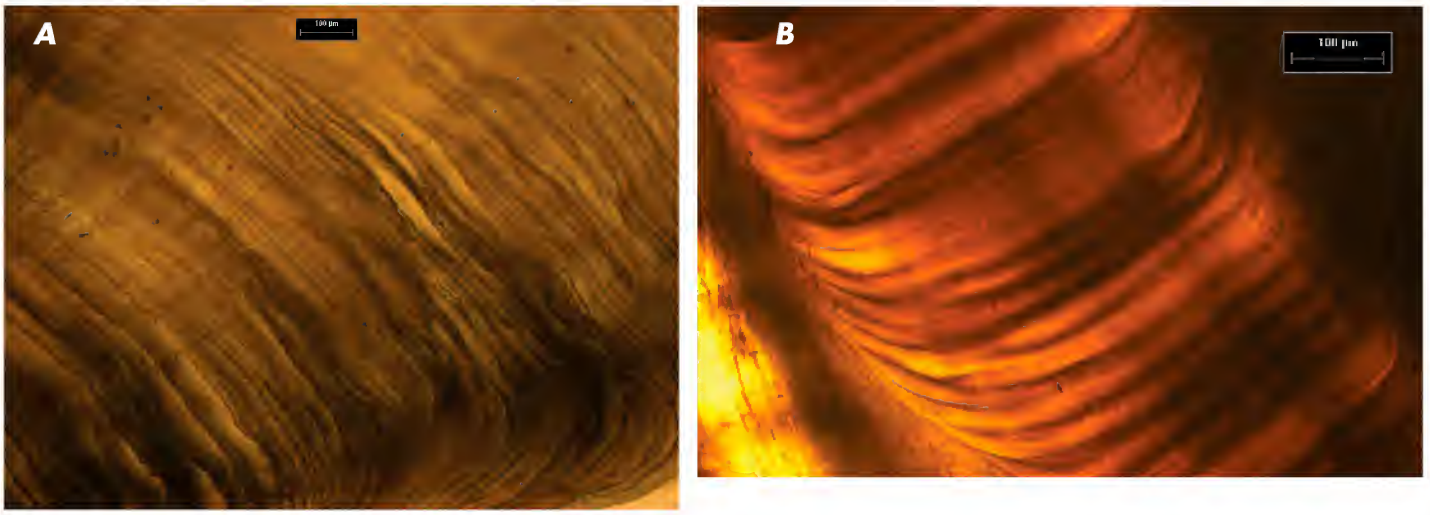

Figure 2. *Photomicrographs of portions of sectioned sagittal otolith microstructure. (A) Misty grouper* Mycteroperca mystacinus *(SEM magnif. X160). (B) Wreckfish* Polyprion americanus *(light magnif. X100). Scale bar = 100 µm.*

TABLE 2. *Maximum total length (TL) and age estimate of medium and large-sized wreckfish (Polyprionidae) and groupers (Serranidae) from the tropical western Atlantic.*

Species (Common name)	**Max. size TL (cm)**	**Max. age (yrs)**	**Reference**
Polyprion americanus (Wreckfish)	192	81	Peres and Haimovici (2004)
P. americanus (Wreckfish)	134	60	present study
Epinephelus flavolimbatus (Yellowedge grouper)	98.5	35	Manickchand-Heileman and Phillip (2000)
	114.8	85	Cook (2007) Cook et al. (in press)
E. guttatus (Red hind)	72	22	Luckhurst et al. (1992)
E. itajara (Goliath grouper)	250	37	Bullock et al. (1992)
E. morio (Red grouper)	90	25	Moe (1969)
E. mystacinus (#1) (Misty grouper)	157	150	present study
E. mystacinus (#2) (Misty grouper)	152	135	present study
E. nigritus (Warsaw grouper)	200	41	Manooch and Mason (1987)
E. niveatus (Snowy grouper)	109	29	Wyanski et al. (2000)
Mycteroperca bonaci (Black grouper)	151.8	33	Crabtree and Bullock (1998)
M. interstitialis (Yellowmouth grouper)	82.7	41	Manickchand-Heileman and Phillip (2000)
M. microlepis (Gag)	116.9	26	Harris and Collins (2000)

Watters et al. 2006) confirm that the ages of large, old fish determined with transverse section microscopy show some underestimation compared to radiometric methods, but the life history interpretation made from the data is the same. Watters et al. (2006) concluded that it is appropriate "to use traditional cross-sectional methods (thin section or break-and-burn) to estimate age for *Sebastes rufus*", as not all fishery scientists have access to radiometric technology. Therefore, we argue that it is better to have a potentially underestimated age estimate for misty grouper than none at all, such that these data can be input into classical methodologies in fishery management, including determination of a von Bertalanffy growth model.

With age estimates of 150 and 135 yrs, our misty grouper specimens appear to be the oldest groupers reported to date in the literature. Interestingly, although the specimens were similar in size, their age estimates differed by 15 yrs. This is possibly due to the fact that as such long-lived species approach asymptotic length, TL will likely increase in very small increments each year. The three largest serranid species, namely, goliath grouper, warsaw grouper and black grouper (Table 2) have reported maximum ages of 37, 41 and 33 yrs, respectively. However, a smaller species, the yellowedge grouper, has been validated with a maximum age of 85 yrs (Cook 2007, Cook et al. in press). The differences in age estimates between misty grouper and the shallow water goliath grouper and black grouper may be a reflection of the deep-water habitat of misty grouper where growth rates appear to be considerably slower (Morales-Nin and Panfili 2005). The reasons for greater longevity in deep-water are uncertain but may be related to altered physiological processes relative to environmental parameters. For example, an analysis of four scorpaenid rockfishes, *Sebastes* spp., indicated that longevity increased exponentially with maximum depth of occurrence (Cailliet et al. 2001), possibly related to low temperature and light levels. Although studies of deep-water, long-lived species have been increasing as these fisheries expand globally (Morales-Nin and Panfili 2005), there is currently insufficient data to determine if such a relationship may hold true for serranids.

In comparison to other deep-water species from different fish families for which there are reliable age estimates, our misty groupers rank amongst the oldest fishes. For example, the maximum age reported for 10 species of slope rockfishes (*Sebastes* spp.; NPFMC 2002) was 140 yrs, with only two species exceeding 100 yrs (Andrews et al. 2002). However, the majority had maximum ages of over 50 yrs. Additionally, age estimates for the orange roughy, *Hoplostethus atlanticus*, using several different aging techniques, have provided maximum age estimates up to 149 yrs (Bergstad 1995), whereas sablefish, *Anoplopoma fimbria* age estimates range up to 70 yrs (Heifetz et al. 1999).

It appears that the age estimates for our misty grouper specimens are broadly consistent with other deep-water teleosts that have been aged. These age estimates extend the maximum longevity of grouper species from the western Atlantic and appear to be amongst the oldest bony fishes aged to date. This lends credence to earlier findings that deep-water fish species can be very long-lived with slow growth rates, characteristics that make them highly vulnerable to even moderate levels of fishing effort (Hopper 1995). Significantly, the capture of such fish, using vertical longline gear at the listed depths, invariably results in 100% mortality. As a consequence, any fishery management measures that are put into effect for deep-water fisheries must account for this factor.

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Note on the Natural and Cultural History of Hurricane Balls

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SHORT COMMUNICATION

NOTE ON THE NATURAL AND CULTURAL HISTORY OF HURRICANE BALLS

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Introduction

Hurricane balls are natural phenomena of tropical storms and hurricane winds and waves and are found along the shoreline. Gunter Library at the Gulf Coast Research Laboratory (GCRL) is home to a small collection of hurricane balls which were found along the shore lines of barrier islands and marsh beaches by GCRL staff over the years. Hurricane balls come in many sizes (Figure 1), and large balls can be slightly larger than a standard basketball of 24.8 cm diameter (USA Basketball 2001). Large balls in the Gunter Library collection range from 34.5 cm diameter (Figure 1A, left side, from 1969 Hurricane Camille) to 53.5 cm diameter (Figure 1A, right side, from 2005 Hurricane Katrina). Small balls range from 4.0 to 11.0 cm diameter (Figure 1B).

Hurricane balls are objects of curiosity, local mythology, and conjecture concerning their origin. Found all over the world and composed of plant fibers native to their coastlines, these objects are called *beach balls*, *drift balls*, *grass balls*, *marsh balls*, *sea balls*, *vegetable balls*, *buffalo balls*, and *whale burps*. However, in south Mississippi, they are called *hurricane balls* (McAtee 1925, Olson 1963, Dubuisson 1969, Clawson 1998, Osis 2000, Ebbesmeyer 2004). This note reviews the scientific and popular literature available about hurricane balls with an emphasis on their cultural and natural history and speculates whether they may be indicators of coastal marsh health.

Hurricane Ball Myths and Local Cultural History

Hurricane balls have been reported from various areas of the United States, both inland and coastal. One of the first reports of the structure is from Ganong (1905), who provided an early review of "certain balls of vegetable matter found on the sandy bottoms of shallow ponds" and cited Thoreau in the classic work *Walden Pond* as finding considerable quantities of curious balls composed of fine grass or roots, perfectly spherical on the sandy bottom of Flint's Pond. Balls that varied from 3.81 to 7.62 cm in diameter washed up along the shores of a lake near Dawson, North Dakota, and were locally called "Buffalo balls" (McAtee 1925). The continual wave action apparently resulted in the formation of balls composed of stems, peduncles and seeds of widgeon grass, *Ruppia maritima*, algae, needles of coniferous trees, debris from chestnut burs, cones of evergreens, and other vegetable substances bound together in a solid firm mass (McAtee 1925).

Osis (2000) refers to similar structures as "beach balls" and reports that they are sold in local gift shops along the Oregon coast as "whale burps," "whale barf balls," or "whale fur balls." However, Osis (2000) dispels the whale regurgita-

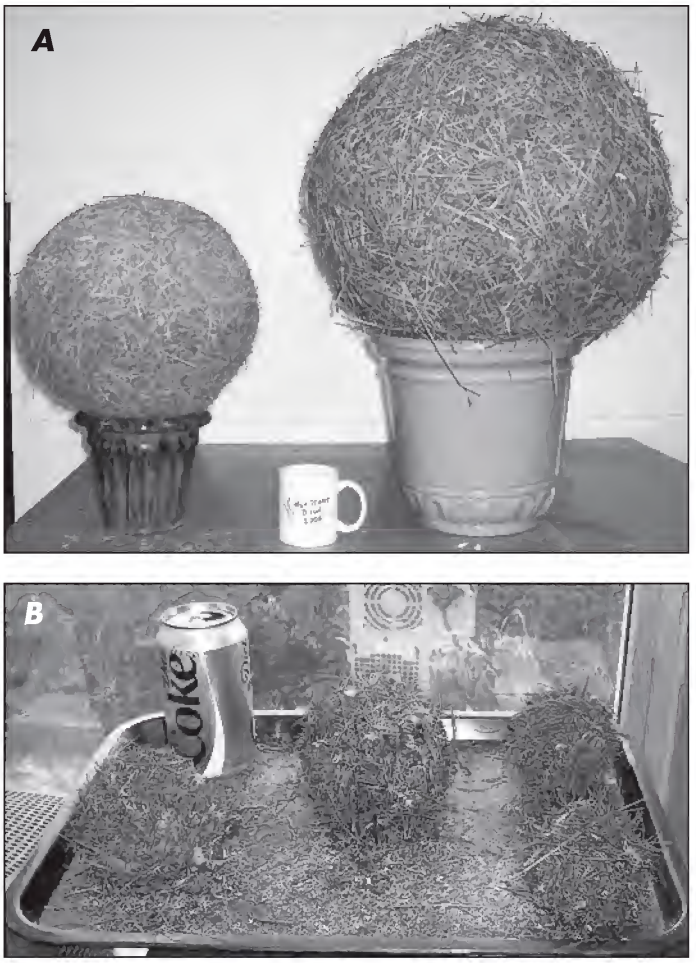

Figure 1. *Hurricane balls housed at the Gunter Library, GCRL, The University of Southern Mississippi. A. The hurricane ball on the left (34.5 cm diameter) was collected after Hurricane Camille in 1969 on Horn Island, and the larger ball (53.5 cm diameter) on the right was found near Weeks Bayou marsh along East Beach Drive in Ocean Springs in spring 2006 after Hurricane Katrina. B. A small collection of different sized hurricane balls (4.0-11.0 cm diameter) found by GCRL staff along the shores of south Mississippi coasts and barrier islands.*

tion myth and describes them as made of vegetation such as sea grass or dune grass mixed with fishing line, snail egg casings, pine needles, seaweed or twigs. The "Betsy balls" displayed at the Audubon Nature Center formerly located east of New Orleans are composed of compacted and woven marsh grass and are named for the 1965 hurricane that made landfall at the mouth of the Mississippi River (Audubon Nature Institute 2004). Finally, Ebbesmeyer (2004) called the thousands of green globs resembling Brillo® pads washed ashore at Plaice Cove, New Hampshire, in June 2002 "sea knitting." He speculated that heavy rains washed nitrates and phosphates from sewage and fertilizers into the ocean, creating an explosion of seaweed growth, and pulsating wave and currents knitted the balls, which consisted of seaweed, sand, and shell fragments (Ebbesmeyer 2004).

Hurricane Ball Natural History

A number of scientists and others report finding hurricane balls such as those seen in Figure 1 during the months following extreme weather events. Along the Mississippi and Louisiana coast and barrier islands, hurricane balls are round or egg-shaped and composed of plant materials such as marsh grasses (*Spartina* spp. and *Juncus roemerianus*) and pine straw formed around a core of plant fibers, roots, or occasionally small pieces of storm debris. The wave and wind action forms a very complex woven structure with plant fibers laced into a sturdy and durable spherical or elliptical object and they can vary in size (Figure 1A and B) but when lifted, they all sprinkle sand.

The formation of hurricane balls was early on speculated to be due to wave action in shallow water on both lake and coastal shores (McAtee 1925). Croneis and Grubbs (1939) compared the formation of siliceous nodules found in the Niagaran dolomite quarries to that of modern "sea balls" and "lake balls." They postulated that the "rolling action of the submerged portions of waves upon the fibrous substances resting lightly upon sandy bottoms" resulted in the genesis of lake balls or sea balls, and while the organic material of the balls may differ, the physical conditions of their formation are similar.

The formation of two sea balls found along the Florida Gulf coast (Olson 1957, 1963) was presumed to be due to wave conditions which tend to work the plant mass into a compact ball-like form. DeVries (1969) speculated that the "spheroidal to ellipsoidal accretionary bodies" composed of dead salt-marsh plants found along groins and jetties in Gulfport, Mississippi, following Hurricane Betsy were formed by heavy surf activity that rolled the floating marsh grass debris into spherical and ellipsoidal masses.

Following Hurricane Camille, GCRL geologist Walter Siler (Figure 2A) found numerous "drift balls" on the shores of Horn and Ship Islands, two barrier islands off the coast of Mississippi, and described them as "accretionary masses of debris formed in very shallow water as a result of wave or current action." Dubuisson (1970) stated that Siler believed the drift balls were composed of marsh grasses from the Louisiana marshlands near Breton and Chandeleur Sounds. These drift balls can be carried by waves and tides throughout the northern Gulf of Mexico with many being concentrated on Mississippi's barrier islands (Figure 2B). Local science educator Leona M. Clawson found about 1,500 hurricane balls along the Mississippi coastal beaches and barrier islands during the months following Hurricane Camille. She set forth a "McCaughan Theory" to describe the mechanical motion of water within waves and their role in forming these objects (Clawson 1998).

Hurricane Balls, Marsh Health, and Barrier Islands

Hurricane balls have been considered to be a negative indicator of marsh health and a warning sign that wetlands and marshes are in distress (Audubon Nature Institute

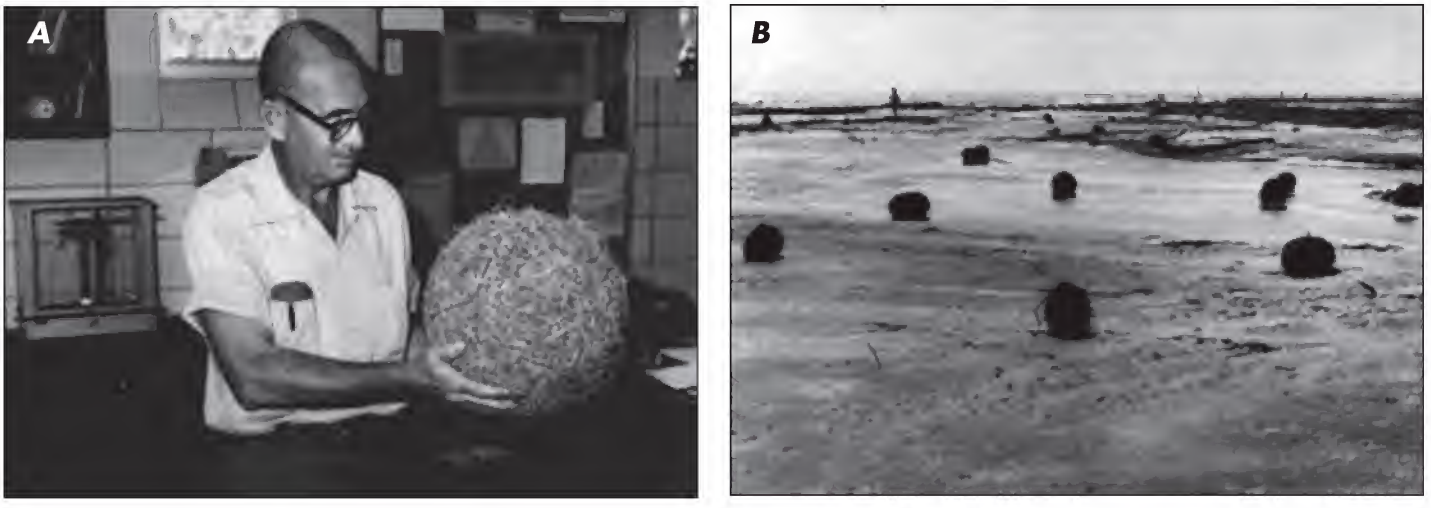

Figure 2. *A. Former Gulf Coast Research Laboratory geologist Walter Siler examines a Hurricane Camille ball. B. Many hurricane balls (sizes not available) washed ashore on Ship Island following Hurricane Camille.*

2004). Hurricanes are known to impact and deform marshes; for example, Hurricane Andrew's impact on the Louisiana marshes included marsh balls created as the marsh was piled, rolled, and deformed (Loveland and McPherson 1998). The marshes on Timbalier Island in Terrebonne Parish, Louisiana, were "stripped of exposed vegetation and the substrate was scoured, and in some areas the marsh was peeled up in strips and formed into 'balls' that were deposited some distance away" during Hurricane Andrew (Penland et al. 2003). This research identified a condition called compressed marsh which is described as a loss of surface area causing the marsh to push together like an accordion. Nearly 25 cm (10 in) of sediment carried by storm surge during Hurricane Andrew was deposited on the floating marsh, causing it to sink (Penland et al. 2003).

Hurricanes Katrina and Rita underscored the fragility of south Mississippi's and Louisiana's barrier islands, coastal marshes, and wetlands (Marris 2005). Louisiana and Mississippi need healthy marshes, sturdy barrier islands, and functioning wetlands to protect their fragile coastlines from the pounding waves and howling winds of tropical storms and hurricanes. However, Landsat satellite data from September and October 2005 showed Hurricanes Katrina and Rita transformed over 100 square miles of marsh to open water, with the most substantial marsh loss occurring east of the Mississippi River in St. Bernard and Plaquemines parishes, Louisiana (U.S. Geological Survey 2005). Hurricane ball formation requires a combination of dead marsh grass, as well as wind and waves from severe weather events. Whether or not the occurrence of these "curious objects" is a warning sign or indicator of coastal and barrier island marshes in distress is a topic of speculation and further research.

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